

MOLECULAR FORMS OF SERUM PROSTATE-SPECIFIC ANTIGEN: THE CLINICAL USEFULNESS OF PERCENT FREE PSA TO DISCRIMINATE PROSTATE CANCER FROM BPH

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The development of increasingly specific diagnostic assays has allowed the detection of various forms of prostate-specific antigen (PSA). It has been found that the proportion of free to total PSA (percent free PSA) is significantly lower in men with prostate cancer than in those with other benign diseases. In order to distinguish early, curable prostate cancer from benign prostatic hyperplasia (BPH), percent free PSA measurement is most useful when the total PSA value is 3–10 ng/ml. The measurement of the proportions of these different forms of PSA, i.e., percent free PSA, may constitute an important diagnostic tool, able to differentiate between benign and malignant prostatic disease with increased specificity, reducing false-positive results and, therefore, improve patient prognosis.

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INTRODUCTION

Although prostate-specific antigen (PSA) is the most clinically useful tumor marker available for the diagnosis and management of prostate cancer¹⁾, currently used PSA testing is insufficiently sensitive and specific for prostate cancer to be considered the ideal tumor marker for early detection of the malignancy. An increased serum PSA level is not pathognomonic of prostate cancer; nor, conversely, do levels below the reference range necessarily indicate its absence. Other conditions, such as bacterial prostatitis, urinary retention, and benign prostatic hyperplasia (BPH) may also be associated with increased serum PSA levels^{2,3)}. To detect prostate cancer with reliability at an early stage, a low serum PSA cutoff level of 4.0 ng/ml is used during screening. However, use of this low cutoff value is associated with an appreciable risk of false-positive results thus diminishing its predictive value and resulting in unnecessary biopsies for those with benign conditions. Approximately 25 to 30% of men with BPH and 80% with proven prostate cancer have serum PSA concentrations above 4.0 ng/ml^{2,4–6)}. Thus, about 20% of men with diagnosed prostate cancer have serum PSA concentrations below 4.0 ng/ml⁵⁾. Earlier studies showed 38 to 48% of patients with clinically significant organ-confined prostate cancer to have normal serum PSA levels^{7,8)}. Had the serum PSA been the sole diagnostic criterion, these men would not have had their cancer diagnosed. The use of a PSA cutoff level of 4.0 ng/ml for screening is associated with a false-positive rate of

65%, and a false-negative rate of 20%, i.e., false-negative results are obtained for men with suspicious findings at digital rectal examination^{4–6)}. The techniques currently used in the immunodetection of serum PSA concentrations are thus of limited clinical value in the early detection of prostate cancer and its distinction from BPH.

In attempts to improve the clinical utility of serum PSA measurements, various concepts have been developed and evaluated, such as PSA density⁹⁾, PSA velocity^{10,11)} and age-specific reference ranges¹²⁾. In addition, several investigations have demonstrated that PSA exists in several molecular forms in serum and that the concentrations of these molecular forms vary according to the disease state of the gland^{13–15)}. The ability to measure the serum PSA level of these specific forms may improve the clinical utility of PSA testing.

BIOSYNTHESIS AND STRUCTURAL SIMILARITY OF PSA AND hK2

PSA is a single chain glycoprotein consisting of 237 amino acids. It is encoded by a gene located at the human glandular kallikrein gene locus on chromosome 19 that also encompasses the genes for tissue kallikrein (hK1) and human prostate glandular kallikrein (hK2 or hGK-1)¹⁶⁾. The expression of PSA and hK2 is androgen-dependent and mainly restricted to the prostatic epithelium, but low-level expression of the two proteins has also been detected in several non-prostatic tissues^{17,18)}.

Owing to the similar pattern of expression of the two proteins and their structural similarity, the

putative immunological cross-reactivity of PSA and hK2 is a critical issue, i.e., the structurally similar human glandular kallikrein 2 (hK2) may cross-react with PSA in assays, thus reducing specificity.

Recently, this issue has been resolved for an extensive panel of anti-PSA antibodies of which a majority of monoclonal anti-PSA antibodies were shown to be exclusively specific for PSA, whereas a limited number of monoclonal and polyclonal anti-PSA antibodies cross-react with hK2¹⁹. Hence, this information can also be used in the design of immunoassays that are specific for PSA alone, or in the design of PSA assays based on cross-reaction with hK2.

DIFFERENT FORMS OF PSA IN EXTRACELLULAR FLUIDS AND IN SERUM

Because PSA is secreted at high concentrations (0.5–5 mg/ml) by the columnar glandular epithelium of the prostate, it is one of the most abundant prostate-derived proteins in seminal fluid²⁰. Approximately 70 percent of PSA in seminal fluid is present in the catalytically active single-chain form^{21,22}. PSA is a serine protease with chymotrypsin-like activity^{21,22}, which is mainly directed towards the major gel proteins in freshly ejaculated semen²¹. A minor proportion (approximately 30%) lacks enzyme activity due to internal cleavage which produces inactive two-chain forms of PSA²². In addition, PSA may be inactivated due to complex formation with protein C inhibitor, alpha-1-antichymotrypsin (ACT) or alpha-2-macroglobulin (AMG)^{22,23}.

The basement membrane of the prostatic acini, the basal cells lining the acini, and stromal cells usually provide efficient barriers ensuring that the serum PSA level does not exceed in serum below 4 ng/ml, corresponding to approximately 10^{-6} of the concentration normally found in seminal fluid. The covalently stable equimolar PSA-ACT complex (approximate molecular size 90 kDa) constitutes the major molecular form of PSA immunodetected in the blood circulation^{13–15,24}. Purified PSA-ACT complex is remarkably stable *in vitro*, a stability further enhanced by the physiological pH (7.4) and the large molecular excess ($>10^{-4}$) of ACT *in vivo*²⁵. A free, non-complexed form of PSA (approximate molecular size 30 kDa) is less abundant, but it has not yet been established whether this occurs in serum in a catalytically active form. This possibility seems unlikely, given the substantial molar excess of ACT and AMG in serum, capable of reacting with an active form of PSA.

IMMUNODETECTION OF THE DIFFERENT FORMS OF PSA IN SERUM

The immunodetection of the different PSA-forms

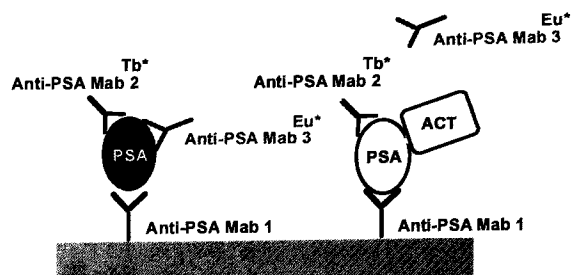


Fig. 1. Schematic illustration of a dual-label immunoassay (Wallac assay, Turku, Finland) for the simultaneous measurement of free and total PSA concentrations and ratios in serum. PSA Mab 1, used for the solid phase capture of PSA, and PSA Mab 2, used as a terbium-labeled tracer antibody, recognize both the free and PSA-ACT complex forms of PSA in an equimolar fashion. PSA Mab 3 (Mab 5A10), used as a europium-labeled tracer antibody, recognizes the free form of PSA only^{19,28}.

may be severely affected by the formation of stable complexes with different protease inhibitors²². Therefore, no antigenic PSA-epitopes are readily exposed by the PSA-AMG complexes and stay undetected by the PSA-assays presently available. The available immunoassays for detection of total PSA in serum (mainly free PSA and PSA-ACT complexes) differ in their capacity to give uniform detection of the PSA forms, in that the signal intensity in the detection of one mole of free PSA equals that of the intensity given by one mole of the PSA-ACT complex; an assay feature most frequently referred to as an equimolar response (Fig. 1)²⁶. The lack of equimolar response results in the report of falsely low total PSA-levels when the proportion of F/T PSA is very low ($<18\%$ free PSA) which is often found in prostate cancer patients^{15,26}. Moreover, the lack of equimolar response also results in the report of falsely elevated total PSA concentrations for $>46\%$ free serum PSA, most often found in men with no prostatic malignancy. Discrepancies in the results reported from different assays for the detection of the total PSA in serum may also be due to whether or not the different antibody combinations cross-react with hK2. Therefore, the clinical utility of hK2 measurements was studied using validated assays²⁷. No hK2 was detected in sera from healthy men and women. Low concentrations of hK2 were detected in sera from patients with BPH, clinically localised prostate cancer and clinically advanced carcinoma of the prostate. The median proportion of hK2 to PSA ranged from 1.1–1.4% in these patient groups, with no significant difference in sera from men with BPH compared with sera from those with localized or advanced prostate cancer²⁷.

MOLECULAR BASIS OF THE LOWER PERCENTAGE OF FREE PSA IN SERUM IN PATIENTS WITH PROSTATE CANCER

It is unclear why the proportion of serum PSA complexed to ACT is higher in patients with prostate cancer than in those with BPH. It does not appear to be related to the concentration of ACT in serum, as ACT is present at 104–105 molar excess to PSA, and the percentage of free PSA does not correlate with the serum ACT concentration^{29,30}. Using immunocytochemistry and in situ hybridization, Bjartell and coworkers demonstrated both PSA and ACT to be produced in the normal glandular epithelium of the prostate³¹. Moreover, a high proportion of cancer cells have been shown to produce both ACT and PSA in most cases of prostate cancer³². In contrast, there was virtually no ACT production in the glandular PSA-producing epithelium in BPH areas³². It is conceivable that, owing to the local production of both PSA and ACT by most prostate cancer cells, conditions are optimal for the formation of PSA-ACT complexes which may then enter the blood circulation. Therefore, owing to the lack of ACT production in the PSA-containing glandular epithelium in BPH areas, conditions are probably less optimal for complex formation, thus facilitating the entry of a free form of PSA lacking protease activity into the blood. However, these findings do not explain, why the percentage of free PSA in serum is also significantly larger in healthy men without detectable prostate cancer, in whose normal prostatic tissue one would expect free PSA to form complex with ACT³³. One explanation may be that the barriers are better preserved in normal than in cancerous prostate tissue, preventing PSA-ACT complexes escaping from the prostate ductal system (and subsequently allowing the percentage of free PSA in serum to be proportionally higher in patients with normal prostates than in those with prostate cancer). However, elucidation of the biologic reason(s) for the difference in percent free PSA levels between patients with prostate cancer and those with BPH will require further studies.

THE CLINICAL UTILITY OF MEASURING DIFFERENT FORMS OF SERUM PSA

Early clinical studies of percent free PSA in prostate cancer detection

The existence of multiple molecular forms of PSA in serum was first reported in 1991^{13,14}. Stenman and colleagues showed the proportion of PSA complexed to ACT to be higher in patients with prostate cancer than in men with BPH¹⁴. The latter group of researchers proposed that the proportion of complexed to total PSA could better discriminate between benign and malignant prostate disease and

reduce the false-positive rate by half.

Independently, Lilja and coworkers demonstrated PSA complexed to ACT to constitute the predominant immunoreactive form of PSA in serum accounting for approximately 85% of total serum PSA, and the free, non-complexed form for the remainder¹³. Subsequently, this group determined the serum concentration of these forms of PSA in patients with BPH or prostate cancer¹⁵. The proportion of free PSA to total PSA (free plus complexed PSA) was shown to be significantly lower in patients with prostate cancer than in those with BPH (0.18 versus 0.28, $P < 0.0001$). This difference was also significant at PSA concentrations below 10 ng/ml. No significant correlation was found between the proportion of complexed-to-total PSA in serum and the total serum ACT concentration. This is not surprising as a large molar excess (104–105 fold) of ACT to PSA exists in serum. The use of measurements of the proportion of free-to-total PSA in serum rather than the total PSA concentration alone was reported to increase the ability of PSA testing to differentiate prostate cancer and BPH from 55 to 73 percent without compromising sensitivity.

These data indicate that knowledge of the concentration and proportions of the different molecular forms of PSA in serum may enable a more reliable differentiation between BPH and early potentially curable prostate cancer. However, the early studies were all based on consecutive archival serum samples from small numbers of patients with wide ranges of total PSA concentrations, varying findings at digital rectal examination (DRE), and different protocols for selecting patients for biopsy.

Confirmatory clinical studies of percent free PSA in prostate cancer detection

Several investigators have now confirmed the early finding that the relative serum concentration of the molecular forms varies according to the disease state of the prostate gland. Although findings in most studies support the notion that free PSA measurements increase the specificity of prostate cancer screening with total PSA, the results have varied considerably. For example, suggested cutoff levels for percent free PSA have ranged from 15 to 25 percent. Similarly, reported figures for the proportion of unnecessary biopsies that can be eliminated by using a percent free PSA cutoff level to select men for biopsy have ranged from 19 to 76 percent^{33–37}. In men with total PSA values of 4 to 10 ng/ml, Luderer and colleagues found the percent free PSA value to differentiate between those with BPH and those with prostate cancer better than total PSA, though the variable was not useful over the whole range of total PSA values³⁷. In most studies, the percent free PSA level has been found to be clinically useful only in men whose total serum PSA concentration is 2.5 to 10 ng/

ml^{34-36,38}). That the clinical utility of the percent free PSA level is best in the "reflex range" of total PSA concentrations of 2.5 to 10.0 ng/ml is logical for two reasons:

- 1) patients with a total PSA level below 2.5 ng/ml are characterized by a very low prevalence of detectable prostate cancer; and

- 2) those whose PSA levels are higher than 10.0 ng/ml are at sufficiently high risk (>50%) to warrant prostatic biopsy, irrespective of the level of percent free PSA.

Several groups of researchers have reported obtaining similar improvements in specificity by using the proportion of free-to-total PSA in serum in the diagnosis of prostate cancer. Both Catalona and coworkers³³⁾ and Luderer and colleagues³⁷⁾, who addressed the utility of percent free PSA within the range of 4.0–10.0 ng/ml, demonstrated the percent free PSA level to significantly improve specificity with a negligible decrease in sensitivity. In 63 men with BPH, 30 men with prostate cancer in an enlarged gland, and 20 with cancer in a normal sized gland, all with total PSA concentrations between 4.1 ng/ml and 10.0 ng/ml, Catalona and coworkers found that the respective median free-to-total PSA proportions to be 0.188, 0.159 and 0.092³³⁾. This was the first study to demonstrate the importance of prostate size in selecting the cutoff value for percent free PSA. In men with a prostate size of less than 40 cc, a free PSA value of 13.7 percent or less detected 90 percent of the cancers and eliminated 76 percent of the negative biopsies. In men with larger glands, a cutoff level of 20.5 percent or less was required to detect at least 90 percent of the cancers, though it eliminated 38 percent of the unnecessary biopsies. Moreover, if the patient had benign findings on digital rectal examination, a free-to-total PSA cutoff of 0.234 or lower was needed to ensure a 90 percent sensitivity of cancer detection. Even at a free-to-total PSA cutoff level of 0.234, 31.3 percent of unnecessary biopsies could have been prevented.

Luderer and coworkers compared 25 patients with prostate cancer and 32 men with BPH, all with total PSA concentrations between 4.0 and 10.0 ng/ml. Based on their data, they constructed theoretical distribution curves of free-to-total PSA proportions of BPH and prostate cancer, and found a cutoff level of 20 percent to have a sensitivity of 80 percent and a specificity of 49 percent³⁷⁾. At a free-to-total PSA cutoff level of 0.25 or less, 31 percent of unnecessary biopsies were prevented, and all of the patients with cancer were correctly identified.

In a highly characterized patient population consisting of 48 men with large benign hyperplastic glands and 51 men with prostate tumor volumes of 2–18 cc, Prestigiacomo and coworkers found that at a cutoff level of 0.15 for the free-to-total PSA proportion

95% (18/19) of the cancer patients were correctly identified as compared to only 54% (14/26) of the BPH patients³⁹⁾. Leinonen and coworkers, who used a double-label time-resolved immunofluorometric assay to simultaneously measure complexed and total PSA in 80 patients with hyperplasia and 44 with cancer of the prostate, found a complexed-to-total PSA proportion of 0.85 to increase the sensitivity of PSA for cancer from 31 percent to 62 percent in patients with values in the range 4–20 ng/ml³⁸⁾.

Recent clinical studies of percent free PSA in prostate cancer detection

Recently studies from several countries, using a variety of different assays, have documented the ability of percent free PSA measurements to improve the specificity of prostate cancer screening without decreasing sensitivity in patients whose total PSA levels are below 20 ng/ml⁴⁰⁻⁴⁸⁾. The proportion of free-to-total PSA in serum is not useful over the whole range of PSA values⁴⁹⁾. This is in agreement with previous findings that the proportion of free-to-total PSA in serum is most helpful in the "reflex range" of 4.0 to 10.0 ng/ml.

One study showed the percent free PSA level to be of help in distinguishing between prostatitis and cancer⁵⁰⁾. Another suggested the variable to be helpful in selecting patients for repeat biopsy (after initial negative biopsies) due to an increased total serum PSA level⁵¹⁾.

Some investigators have suggested probability or risk ranges for percent free PSA to be more useful than arbitrary cutoff levels^{52,53)}. However, for probability ranges to be valid, the prevalence of prostate cancer should be the same in the study population as it is in the target population, which was not the case in some reported studies⁵²⁾.

In a recent study by Björk and coworkers, the diagnostic power of several assays was compared, using receiver operation characteristics (ROC) to calculate the areas under the curve (AUC) for the dual-label measurements of free and total PSA, and the optimized PSA-ACT complex measurements in 47 patients with BPH and 39 with localized prostate cancer⁵⁴⁾. All 86 patients included in the study had total PSA levels in serum from 1.0–20 ng/ml. The F/T-PSA measurements manifested the most outstanding diagnostic power, but the AUC for these measurements were only slightly higher, and not statistically significantly different, from those of the free PSA to PSA-ACT complex measurements. However, the free-to-total PSA measurements had significantly higher diagnostic power than those of all other assays alone, or combinations of assays. In Japan, Higashihara and coworkers evaluated the significance of free PSA in mass screening of prostate cancer at Mitaka area of Tokyo. With the use of Hybritech assay, they obtained 50% of positive

predictive value with 60% of avoidance of unnecessary biopsy. Their optimal cutoff value of F/T was 12%⁵⁵⁾

The optimal total PSA range for measuring percentage free PSA (reflex range) was defined in a study in 'at risk' subjects⁵⁶⁾. Percentage free PSA measurement was found to be most useful when the total PSA value was 3–10 ng/ml. Its ability to distinguish early, curable cancer from benign prostatic hyperplasia (BPH) over this range was superior to total PSA by >20%.

The clinical utility of the F/T-PSA testing is now being further evaluated in large scale population based prospective studies on the efficacy of early detection and treatment of prostate cancer (see "Prospective studies of percent free PSA in men at risk for developing prostate cancer").

STUDIES FAILING TO SHOW PERCENT FREE PSA TO BE A USEFUL VARIABLE

Other studies have shown the percent free PSA level not to be clinically useful in screening^{36,49)}. Bangma and coworkers, using the Wallac assay, found the percent free PSA level to improve the specificity of testing, but the improvement was non-significant and the variable was of no use in men whose prostate was smaller than 50 cc³⁶⁾. However, this series included men with PSA values ranging from 0.2 to 150 ng/ml, and the utility of percent free PSA measurements was evaluated only by comparing the area under ROC (receiver operating characteristic) curves (plots of sensitivity versus 1 minus specificity). On the other hand, had a cutoff value of 28 percent been used for the proportion of free PSA in the study by Bangma and coworkers, where the range of total serum PSA levels was 4–10 ng/ml, ≥90 percent sensitivity could have been maintained while eliminating 19 percent of unnecessary biopsies in the series as a whole and 49 percent in the subgroup with prostates smaller than 50 cc.

In the Baltimore Longitudinal Study on Aging, Pearson and colleagues found percent free PSA levels to decline progressively in patients with prostate cancer several years before the diagnosis of cancer was established⁵⁷⁾, the mean value decreasing from approximately 22 percent several years before the diagnosis of cancer to about 15 percent at the time of diagnosis. This suggests that the progression of prostate cancer from the preclinical to the clinical phase of the disease is reflected by a decline in percent free PSA, which in turn suggests relationship to exist between the percent free PSA level and the potential biologic aggressiveness and extensiveness of prostate cancer, as reported by Arcangeli and coworkers⁵⁸⁾ (see below).

Kuriyama compared F/T, prostate volume and PSAD in screening prostate cancer in Japanese who

showed 4–10 ng/ml of total PSA. Number of cases evaluated were 77 of prostate cancer and 266 of BPH. He reported that prostate volume (40 or 45 cc) and PSAD (0.15) showed >40% of specificity maintaining >90% of sensitivity, which was better result than those of total PSA (5.0 ng/ml) or F/T(0.25). Although F/T values were found a statistically significance in their distribution between prostate cancer and BPH, he concluded F/T may not be the first line method for screening prostate cancer⁵⁹⁾

PROSPECTIVE STUDIES OF PERCENT FREE PSA IN MEN AT RISK FOR DEVELOPING PROSTATE CANCER

The true value of using the proportion of free-to-total PSA in a clinical setting will be known when large ongoing screening studies have been completed^{60,61)}. Bangma and coworkers found 67 prostate cancers in 1726 screened men all investigated with DRE, TRUS and serum PSA. Using cutoff levels of 4.0 ng/ml for total serum PSA, and 0.20 for the proportion of free-to-total PSA and/or an abnormal DRE, they would have been able to reduce the number of biopsies with 39 percent at a cost of 11 percent missed cancers⁶¹⁾

Physicians usually recommend biopsies only in men whose PSA levels are higher than 4 ng/ml. However, there is accumulating evidence that prostate cancer is detected within 3 to 5 years in 13 to 20 percent of men whose total PSA levels are between 2.6 and 4 ng/ml^{34,62)}. Detecting these cancers earlier without markedly increasing the number of unnecessary biopsies would enable more men to seek treatment before their cancer has spread. In this regard, about 30 percent of men with PSA levels between 4 and 10 ng/ml have cancers characterized by extracapsular extension or metastasised at the time of diagnosis⁴⁻⁶⁾

Dr. Catalona and coworkers reported on retrospective and prospective series of consecutive screening volunteers whose total serum PSA levels were 2.5 to 4.0 ng/ml⁶³⁾. In the first study, the researchers evaluated whether percent free PSA (Hybritech) could predict cancer in men with either abnormal or suspicious findings at rectal examination or prostatic ultrasonography and total PSA levels of 2.8 to 4 ng/ml. They reviewed the medical records of 120 men with initial PSA levels of 2.8 to 4 ng/ml who had undergone biopsies as part of a pilot study; prostate cancer was detected in only 7 percent. However, during the next four years, cancer was detected through serial screening in an additional 15 percent. Catalona and coworkers measured percent free PSA in the patients' initial frozen blood samples and found that, using a cutoff level of 23 percent free PSA, the test could identify 93 percent of the men with cancer and would have avoided 28 percent of

unnecessary biopsies. Catalona and coworkers confirmed these findings in a prospective study of 217 consecutively screened volunteers whose total PSA levels were 2.6 to 4 ng/ml and whose rectal examination findings were not indicative of cancer. A cutoff of 25 percent free PSA would have detected 91 percent of the cancers, avoided 26 percent of the negative biopsies, and yielded a positive predictive value of 19 percent.

These results suggest that percent free PSA should be measured in all men with serum PSA levels of 2.5 to 10 ng/ml. Those with a serum PSA level of 2.5 to 4 ng/ml and less than 25 percent free PSA could then decide whether to undergo biopsy or wait until the total serum PSA level exceeds 4 ng/ml. Lowering the recommended serum PSA level to 2.5 ng/ml for biopsy might be especially beneficial for black men whose incidence of prostate cancer is nearly 40 percent higher than in white men⁶⁴⁾. Blacks are also more likely to have more advanced prostate cancer at the time of diagnosis, and are more likely to die from the disease. However, the potential benefit of increasing the early cancer detection rate should be weighed against the increase in the number of biopsies required. Using percent free PSA should help reduce the number of unnecessary biopsies.

At the American Urological Association Annual Meeting in New Orleans, 1997, Dr. Catalona and coworkers presented an on-going multicenter clinical trial designed to establish guidelines for use of percentage free PSA⁶⁵⁾. It is the largest series to date of subjects with PSA 4–10 ng/ml and histologically-confirmed diagnoses of prostate cancer. In total, 622 subjects have been enrolled to date at 7 medical centers in the United States. Male subjects, 50–75 years of age, with nonsuspicious rectal examination, PSA 4–10 ng/ml, underwent sextant biopsies that resulted in 301 patients with prostate cancer and 321 cases of BPH.

Preliminary study results showed that percent free PSA increased the clinical specificity of PSA. Cutpoints producing 95% or 90% sensitivity allowed a 20% or 30% decrease in negative biopsies respectively. In order to maintain constant sensitivity, cutpoints decreased as PSA increased: the 95% sensitivity cutpoint changed from approximately 28% to 24% as PSA rose from 4 to 10. In contrast, percent free PSA increased as patient age and prostate volume increased. Percent free PSA is an independent predictor of prostate cancer, and contributed significantly more than age or PSA 4–10 ng/ml. Lower percent free PSA values corresponded with higher risk of cancer. Percent free PSA <10 was associated with 59% probability of cancer, whereas >26 percent free PSA was associated with 10% probability of prostate cancer.

COMPARISON OF FREE PSA WITH OTHER METHODS OF IMPROVING ACCURACY OF PSA TESTING

It is important to consider how percent free PSA compares with other methods of increasing the specificity of prostate cancer screening. The three other methods that have been proposed are PSA velocity^{10,11)}, PSA density^{9,66)}, and age-specific reference ranges¹²⁾.

PSA velocity (PSAV) is defined as the change in serum PSA over time¹⁰⁾. PSA velocity is limited by the intrinsic biologic variability (5 to 15 percent) of PSA testing and the fact that time, multiple measurements, and the same assay kit are required to measure velocity accurately⁶⁷⁾. A recent study concluded that for men with a normal digital rectal examination (DRE) and a PSA value of 4–5 ng/ml, a two year PSA testing interval was adequate for maintaining the detection of 'curable' prostate cancer⁶⁸⁾.

PSA density (PSAD) is defined as total serum PSA (ng/ml) divided by prostate gland volume (cm³)⁹⁾. It is based on the assumption that the PSAD calculation would adjust for the portion of PSA resulting from BPH, providing a reference value above which further investigation is required. The use of PSAD in different patient populations for diagnostic and staging purposes has demonstrated its usefulness and cost effectiveness as a second line screening parameter, helping to distinguish benign conditions from prostate cancer in patients with an intermediate PSA level. However, PSAD is limited by the inaccuracy of ultrasound measurements of prostate volume and the invasiveness and cost of ultrasonography.

Age-specific reference ranges is defined as adjusting the upper normal limit of the serum PSA level according to patient age¹²⁾. Serum PSA increases with age¹²⁾, at a rate of around 0.04 ng/ml/year in populations free from cancer, and also varies with race⁶⁹⁾. The age specific reference ranges for different races are shown in Table 1.

Use of the recommended age-specific reference ranges has been criticized by Catalona and colleagues owing to the indiscriminate increase in the rate of unnecessary biopsies in men under 50 years of age and

Table 1. Age-specific reference ranges for serum PSA. Reprinted from reference 67. Copyright 1993, American Medical Association

Age Range (years)	Reference Range (ng/ml)		
	Asians	Blacks	Whites
40–49	0.0–2.0	0.0–2.0	0.0–2.5
50–59	0.0–3.0	0.0–4.0	0.0–3.5

the delay in detection of curable cancers in men aged 60 to 73 years who may be legitimate candidates for curative surgery. Moreover, the mean (plus 2 standard deviations) for a given age group is not necessarily the best cutoff level to use for cancer screening. Finally, PSA 'leakage' into the serum does not always increase with age⁷⁰⁾. However, no normal comparisons of percent free PSA with PSA velocity and age-specific reference ranges have been published, though Smith and colleagues who compared percent free PSA with PSA density in the cohort of men previously studied by Catalona and coworkers³³⁾, demonstrated both variables to be significant independent predictors of cancer after controlling for age, total PSA, and rectal examination results⁶⁴⁾. Smith and colleagues also showed both variables to improve specificity by about 38 percent, though the PSA density cutoff level had to be lowered from the standard 0.15⁶⁶⁾ to 0.09 to maintain sensitivity at 90 percent, and a cutoff level of 20.3 percent free PSA to maintain 90 percent sensitivity in men with total PSA levels of 4.1 to 10 ng/ml. The use of percent free PSA would be more cost effective than PSA density for screening purposes because it does not require the performance of ultrasonography in all patients.

RELATIONSHIP BETWEEN PERCENT FREE PSA AND AGE, PROSTATE VOLUME, AND TOTAL PSA

Conflicting information has been published on the relationships between patient age, the absolute concentration of free PSA and the percent free PSA level. The appropriate upper normal limit of the free-to-total PSA proportion has been concluded to be constant irrespective of age²⁴⁾. Although this might be interpreted as implying that no corrections need to be made for age, the data nonetheless suggested weak, but statistically significant correlation ($p=0.02$) to exist between age and both the free-to-total PSA proportion and the complexed-to-total PSA in serum²⁴⁾. The serum concentrations of each molecular form of PSA (free PSA, complexed PSA, and total (free+complexed PSA) PSA) have also been studied in a randomly selected group of 422 healthy Japanese men, 40 to 79 years of age²²⁾. The serum concentrations of each molecular form correlated directly with age and the volume of the prostate gland.

There have also been conflicting reports on the relationship between the level of free PSA and prostate volume. Yemoto and colleagues found no correlation to exist between prostate volume and the absolute free PSA concentration or the percent free PSA value⁴⁸⁾.

Data from several studies suggest inverse relationship to exist between percent free PSA and

total PSA^{15,34,36)}. This relationship suggests that men with higher PSA levels (and with more aggressive or advanced disease) have lower percentages of free PSA. Thus, age, prostate volume, and the total PSA concentration are important factors that need to be taken into account in selecting the cutoff level for percent free PSA.

RELATIONSHIP BETWEEN THE PERCENT FREE PSA LEVEL AND PATHOLOGIC FEATURE

Arcangeli and coworkers have published a preliminary report of a study in which the percent free PSA helped distinguish between potentially aggressive and non-aggressive prostate cancers⁵⁸⁾. In this study of 54 consecutive men with prostate cancer detected at screening who underwent radical prostatectomy, they found a low percent free PSA level to be associated with potentially more aggressive tumors. In this group of patients with total PSA levels ranging from 2 to 14 ng/ml (>90% were between 2 and 8 ng/ml), a higher total PSA was not significantly associated with adverse pathologic features of cancer, and a lower total PSA value was not predictive of "insignificant" cancers. However, a lower percent free PSA level was significantly associated with adverse pathologic features in the tumors-eg. extracapsular extension, cancerous surgical margins, a higher Gleason score, and a greater tumor volume. Using an arbitrary cutoff level of 20 percent free PSA would have identified 90 percent of the tumors with adverse features. They also found percent free PSA values above this cutoff level to be associated with potentially insignificant cancers.

In contrast, other investigators have found no significant correlation to exist between percent free PSA and tumor stage⁷¹⁻⁷⁴⁾, though most of these studies included patients with high total serum PSA levels (some with levels in the 40 to 50 ng/ml range). Further research is needed to clarify this issue. If these results are duplicated in a large number of patients and by other investigators, the percent free PSA may help physicians and patients decide which tumors should be treated and which can be safely left alone.

SUMMARY

The concept of measuring the proportions of various forms of PSA in serum, particularly the proportion of free to total PSA, represents a new and exciting method of detecting early curable prostate cancers while avoiding unnecessary prostate biopsies in men who have BPH only. As compared to other methods of improving diagnostic specificity, it does not require transrectal ultrasound for determination of prostate volume, as does the use of PSA density,

and it does not require multiple blood sampling over a sufficiently long period, as does PSA velocity.

Recent findings suggest determination of the proportion of free-to-total PSA, i.e., percent free PSA, to be the optimal discriminator of patients with prostate cancer and those with BPH when the total PSA value is 3–10 ng/ml, and to improve the clinical accuracy of the PSA test substantially. This might enable considerable reduction in the number of confirmatory tests required in a screening setting, with a consequent reduction in morbidity and costs.

In conclusion, percent free PSA may constitute an important diagnostic tool to be used as a guideline in detecting early prostate cancer and to have its greatest clinical impact when the total PSA value ranges from 3 ng/ml at the low end to 10.0 ng/ml at the high end. However, many unanswered questions about percent free PSA need to be investigated. Thus, percent free PSA should still be considered an investigational concept in 1997.

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