Prostate-Specific Antigen: Current Status

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Abstract

Prostate-specific antigen (PSA) is the most important of all tumor markers in that it has significant applications in all aspects of the management of men with prostatic disease. Certainly, the most important utilization of PSA is for the early detection of this most ubiquitous of all human neoplasms.

This article reviews the salient features of PSA, with particular emphasis on strategies to improve its utility in the diagnosis of prostate cancer. So-called PSA derivatives—including age-specific PSA, PSA velocity, and PSA density—are discussed. With the recognition of molecular forms of PSA, however, the ratio of free-to-total PSA, and now the complex form of PSA, have been shown to be more specific indicators of the presence of malignancy.

Significant public interest and research efforts in prostate cancer have resulted in numerous advances over the past decade. The discovery of PSA and the development of assays to measure it will undoubtedly be recorded as one of the most important advances in the management of men with prostate cancer. (CA Cancer J Clin 1999;49:264-281.)

Introduction

Prostate cancer remains the most common male malignancy and the second most common cause of prostate-related mortality in the United States.1 Many researchers have expended considerable efforts to alter these sobering statistics.

In all significant diseases, three opportunities exist to decrease mortality. These include decreasing the incidence of disease, improving treatment, and facilitating early detection.

A number of investigations are underway to lower the incidence of prostate cancer. Ultimately, this will require a clear understanding of the etiology of the disease. In the interim, however, several chemopreventive strategies have been initiated, the most ambitious of which is the Prostate Cancer Prevention Trial in which men are randomly assigned to receive the 5-alpha reductase inhibitor finasteride or placebo for seven years. The study has completed accrual, but it will be several years before the results are known.

Major strides have been made in therapeutic modalities for clinically localized prostate cancer. Improvements in surgical technique, as well as in radiation methodologies and novel therapeutic approaches, have all decreased the morbidity associated with the disease. Unfortunately, the most common cause of death from prostate cancer continues to be hormone refractory metastatic disease. While research efforts continue in an attempt to develop novel therapeutic strategies for this stage of disease, it is unlikely that significant improvements in efficacy will be realized soon.

The third option for lowering mortality associated with any cancer is to enhance early detection. Three major technologies, together with increased public awareness about cancer in general and prostatic disease in particular, have dramatically changed the incidence of

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This article is also available at http://www.ca-journal.org.
prostate cancer in the United States and other developed nations. Transrectal ultrasound, spring-loaded biopsy devices, and most importantly, serum assays to measure prostate-specific antigen (PSA) have revolutionized screening and early detection of this ubiquitous neoplasm.

This article will review the clinical utility of PSA for diagnosis and screening. Although the role of this analyte for staging and/or monitoring of established disease will not be covered here, several recent review articles are available.2-4

**PSA: In the Beginning**

While many have claimed to be the first to discover PSA in seminal plasma, most authorities credit Wang and associates, working at the Roswell Park Cancer Institute in Buffalo, NY, in conjunction with Murphy et al, also at Roswell Park at the time, as having clearly described this protein on gel electrophoresis.5 The first definitive clinical study, conducted by Stamey and associates at Stanford University, investigated this analyte in a number of settings.6

While recognizing PSA’s importance in monitoring established disease and staging, these and many other early investigations suggested that PSA would be of limited utility for screening or early detection owing to a lack of specificity (Table 1).6-9 Many studies clearly demonstrated that between 21% and 90% of patients with established benign prostatic hyperplasia undergoing prostatectomy had abnormal levels of PSA. Given that virtually every man who undergoes testing for prostate cancer has at least microscopic evidence of benign prostatic hypertrophy, the utter lack of specificity was felt to render this test useless in a diagnostic setting.

We began to question this conclusion, however, on the basis of two findings. In an effort to understand which pathologic features might be associated with elevated PSA levels, we performed a detailed analysis of all resected or enucleated tissue removed from men undergoing simple prostatectomy for presumed benign prostatic hyperplasia.10 While approximately 50% of those men with incidental carcinoma, acute inflammation, or prostatic intraepithelial neoplasia (the premalignant change) had serum PSA levels greater than 4.0 ng/ml, only one of 26 men with benign prostatic hypertrophy alone or hypertrophy associated with chronic inflammation exceeded this threshold.

**MECHANISM OF ELEVATED PSA**

These observations suggested a mechanism underlying elevated PSA and prompted us to consider the relationship of the pathology in the prostate and elevated PSA.

The prostatic lumen contains the highest concentration of PSA in the body. Significant barriers, including the prostate basement membrane, the intervening stroma, the capillary basement membrane, and the capillary endothelial cell,
are interposed between the prostatic lumen and the capillary blood (Fig. 1). Several of these protective layers may be broached when disease processes, e.g., prostate cancer, prostatic intraepithelial neoplasia, and prostatitis, are present.10-12 We hypothesized, therefore, that specific disease processes not noted in the older series, (see Table 1), might indeed explain elevated PSA levels.

The second piece of evidence that prompted us to begin to consider and evaluate PSA for screening and early detection emerged indirectly. Independent reports by the late William Cooner,13 as well as by our own group,14 revealed striking findings when serum PSA was measured in men undergoing ultrasound-guided prostate biopsy (Table 2). In his investigation, Dr. Cooner selected patients to undergo biopsy if the ultrasound revealed a hypoechoic peripheral zone lesion. In our own investigation, the indication for biopsy was an abnormality on digital rectal examination. While PSA media announcements and offered a free PSA test. If the PSA was greater than 4.0 ng/ml, ultrasound-guided systematic sector prostate biopsy was recommended.15 An almost identical study design was carried out by Catalona and associates at Washington University in St. Louis. The salient findings from these two investigations are presented in Table 3.16, 17 These two studies clearly demonstrate that a PSA greater than 4.0 ng/ml in a man 50 years of age or older was associated with an approximately 33% chance of detecting prostate cancer on the initial diagnostic biopsy. These findings have since been confirmed by many other researchers (Table 3).13, 14, 16-24

Clinically Significant Cancers

PSA not only detects a significant number of prostate cancers, but also the cancers detected are generally of clinical significance.14, 17, 19-21, 25 This analyte has truly revolutionized our approach to screening and early detection of prostate cancer.

PSA FOR EARLY DETECTION

On the basis of these findings, in 1990, we initiated a PSA early detection program in the Puget Sound region. Men were recruited through a variety of public
Table 2
PSA level as an Indicator of Prostate Cancer: Early Experience

|                | PSA > 4.0 ng/ml |            | PSA > 10.0 ng/ml |            |
|----------------|----------------|------------|------------------|------------|
|                | Brawer et al\textsuperscript{14} | Cooner et al\textsuperscript{21} | Brawer et al\textsuperscript{14} | Cooner et al\textsuperscript{21} |
| Number of Patients | 188 | 96 | 188 | 96 |
| Number of Patients with Cancer | 78 | 28 | 77 | 28 |
| (41.0%) | (29.2%) | (41.0%) | (29.2%) |
| Sensitivity | 67.5 | 75.0 | 41.6 | 35.7 |
| Specificity | 60.3 | 70.6 | 82.0* | 94.1* |
| False Positive | 39.6 | 29.4 | 18.9* | 5.9* |
| False Negative | 32.5 | 25.0 | 58.4 | 64.3 |
| Positive Predictive Value | 54.2 | 51.2 | 60.4 | 71.4 |
| Negative Predictive Value | 72.8* | 87.3* | 66.7 | 78.1 |

*Statistically significant difference (p < 0.01)

Table 3
Correlation of PSA Level Greater than 4.0 ng/ml and Cancer: Confirmatory Studies

| Author                  | Year | Population | Number of Biopsies | PPV |
|-------------------------|------|------------|--------------------|-----|
| Babaian and Camps\textsuperscript{18} | 1991 | Mixed | 67 | 31.3 |
| Bazinet et al\textsuperscript{19} | 1994 | Referral | 565 | 37 |
| Brawer and Lange\textsuperscript{14} | 1989 | Referral | 188 | 54.2 |
| Brawer et al\textsuperscript{17} | 1992 | Screening | 105 | 30.5 |
| Catalona et al\textsuperscript{16} | 1991 | Screening | 112 | 33 |
| Catalona et al\textsuperscript{20} | 1994 | Screening | 1,325 | 37.1 |
| Cooner et al\textsuperscript{21} | 1988 | Referral | 96 | 51.2 |
| Cooner et al\textsuperscript{13} | 1990 | Referral | 436 | 35 |
| Ellis et al\textsuperscript{22} | 1994 | Referral | 541 | 36.8 |
| Mettlin et al\textsuperscript{23} | 1994 | Screening | 70 | 41.4 |
| Rommel et al\textsuperscript{24} | 1994 | Referral | 2,020 | 41 |

PPV=Positive Predictive Value
cancer, a statement that is underscored by the fact that PSA is the only serum analyte approved for diagnostic purposes by the United States Food and Drug Administration. Moreover, both the American Cancer Society and the American Urologic Association have recommended annual serum PSA determination in combination with a digital rectal examination in men presenting for cancer prevention check-ups.26, 27

**Drawbacks of PSA Testing**

Despite these impressive results, PSA is not the ideal tumor marker. Considerable efforts are underway to improve performance, with emphasis directed primarily at enhancing specificity by reducing false-positive test results. The slow growing nature of prostate cancer, and the fact that it is likely that a man will be tested more than once in his lifetime, makes a false-negative test less important. If the PSA level, in the presence of malignancy, is below the threshold indicating the need for further evaluation, it is likely that repeat testing will occur and the cancer will be detected at a time when it is still curable (Fig. 2). False-negative results in this setting, therefore, are generally considered less important.

In marked contrast, false-positive test results are costly, both financially (ultrasound-guided biopsy, pathology charges, etc., result in a total per-person expense of approximately $1,500 dollars) and emotionally. Patients and families often suffer considerable anxiety when informed of an abnormal PSA result. Thus, the major efforts in PSA enhancement have focused on reducing false-positive results (i.e., enhancing specificity).

It must be remembered, however, that, in general, whenever efforts are made to enhance the specificity of a diagnostic test, the sensitivity (i.e., the identi-
fication of patients with the disease in the population) is reduced (Fig. 3). Figure 3 is derived from a large ultrasound-guided prostate needle biopsy experience in which the sensitivity and specificity for differing PSA levels were determined. As is readily apparent, increasing the PSA threshold will enhance specificity but does so at the expense of sensitivity. In effect, what we must consider is how many cancers are we willing to miss to avoid biopsy in men without malignancy? While no clear answer is appropriate for all patients, and there is considerable debate among prostate cancer experts, most authorities are targeting a goal of approximately 95% sensitivity.

Enhancing PSA Performance

A number of the modalities have been proposed to enhance PSA performance (Table 4).

PSA Velocity

Measuring PSA velocity, or the change of PSA level over time, certainly makes intrinsic sense. In men who progress from benign disease to advanced prostate cancer, there is invariably an elevation in the PSA level, and almost always, this increase exceeds the generally slow rise in PSA observed in healthy men over time. Carter and associates at Johns Hopkins University were the first researchers to capitalize on this observation. These authors noted that if a man’s PSA rose at a rate greater than 0.75 ng/ml per year, he was at increased risk of being diagnosed with prostate cancer. A number of clinicians have attempted to expand on this finding and have applied the concept of PSA velocity in patients followed at intervals as short as one year or even less. It is important to note, however, that in the Johns Hopkins study, PSA determinations were separated by a minimum of seven years.

We were unable to reproduce the results of Carter et al with one- and two-year intervals between PSA determinations. In an effort to understand our results, we examined the biologic variation of PSA on a daily basis for 10 consecutive days. These studies by Nixon et al demonstrated that before a change in PSA could be ascribed to a real prostatic disease process, a 25% increase must be observed. This calculation takes into account biologic variation, as well as laboratory issues. For free PSA, an even greater change of 36% must be noted (Table 5).

PSA Density

PSA density is a derivative measure that involves dividing the serum PSA level by the volume of the prostate. This is an attempt at enhancing PSA specificity by adjusting for that component of the serum PSA that may arise from benign elements. The largest determinant of prostate size is the transition zone, with expansion resulting from the development of benign prostatic hypertrophy.

Babaian and associates first called attention to the relationship between prostate size and serum PSA level; subsequently, Benson and associates at Columbia University promulgated the notion of PSA density. While this, too, makes some intrinsic sense, we were again unable to confirm their findings. Indeed, in our experience, we showed that PSA alone was as good a predictor of carcinoma as PSA density. A possible explanation for this discrepancy is illustrated in Figure 4. The diagram on the left depicts an 80-cubic centimeter prostate in a man with a serum PSA level of 8 ng/ml. His PSA density would be 0.1.

The figure on the right shows the prostate in a man with the same serum PSA level but a gland half as large as his counterpart on the left. With a PSA density of 0.2, one might assume that carcinoma was more prevalent in this man than in the subject on the left. If both men have a 1-centimeter isoechogenic non-palpable (T1c) carcinoma, and a standardized number of prostate biopsies are obtained from both
glands, it is more likely that sampling constraints will cause us to miss the carcinoma in the larger gland while identifying it in the smaller (Table 6). In the studies that demonstrated enhanced efficacy of PSA density compared with PSA alone, there was a concomitant finding that larger prostates showed benign histology. In contrast, the studies by Brawer and Mettlin showed either no difference between glands with malignancy and those without, or in the case of Mettlin, that cancer-containing glands were actually slightly larger. More recent data from our group have demonstrated, in fact, that the yield from prostate needle biopsy is reduced in larger glands (Fig. 5). Rather than suggest that larger prostates afford some degree of cancer prevention, we feel that this is further evidence of the sampling limitations of current biopsy methodology. Further work is needed in this area.

In an effort to re-examine the role of PSA density, a number of investigators have studied the transition zone density. Transition zone density is defined as the serum PSA divided by the volume of the transition zone—again, usually measured by transrectal ultrasound. Although the initial reports were encouraging, we have been unable to reproduce these data in our series (Fig. 6). In our experience, no difference was noted in the ability of PSA, PSA density, or transition zone density to stratify men for the presence of cancer (Fig. 7). As with PSA density, sampling bias may be a limiting factor with respect to transition zone density.

The next approach to enhancing PSA performance, the so-called age-adjusted PSA, refers to the idea that the use of a single cutoff level for all ages is inappropriate. It is well recognized that PSA increases with age. Both Oesterling and associates and Dalkin et al suggested different cutoffs for different age groups. Thus, according to Oesterling, a man younger than 50 should have a PSA below 2.5 ng/ml, whereas for a man in his 70s, a PSA between 0 and 6.5 ng/ml might be considered normal. While this is a true observation based on men apparently free of prostate disease, utility in screening and

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**Figure 3**

PSA and Sensitivity/Specificity

The inverse relationship of PSA sensitivity and specificity for men undergoing ultrasound-guided prostate needle biopsy. Data from 2,800 consecutive prostate needle biopsies.
early detection is unclear.

It is certainly true, as illustrated in Figure 7, that the biopsy yield (positive predictive value) increases with increasing PSA level. What is also true, of course, is that the detection of cancer increases as men age. Thus, age-adjusting PSA states the obvious, namely that PSA and prostate cancer increase with age. If our goal is to have the same yields for cancer in each age group, then this adjustment in the PSA level makes sense. If our goal, on the other hand, is to detect the greatest number of cancers, we should preferentially test the older patients because they have a higher prevalence of disease. Some have argued, however, that finding cancer in younger men is more clinically significant. In an effort to address these concerns, we studied the age-adjustment PSA cutoff versus 4.0 ng/ml (the most widely utilized cutoff), based on our PSA screening cohort.

As shown in Table 7, the number of men exceeding a threshold in a screening of men older than 50 years of age, as well as the positive predictive value, is slightly enhanced with age-specific PSA. However, the detection rate (i.e., the number of cancers identified in the screening population) is significantly reduced with these cutoffs. Moreover, based on the United States, the economic implications of these strategies are significant.

Table 4
Strategies for Enhancing PSA Specificity
- PSA Velocity
- PSA Density
- PSA Transition Zone Density
- Age-Specific PSA
- Free/Total PSA Ratio
- ACT* Complex PSA

*ACT=Alpha-1 antichymotrypsin

Table 5
Daily Variation of PSA Level (mean results)

| Category                  | Free PSA | Total PSA | % F/T PSA |
|---------------------------|----------|-----------|-----------|
| Biological variation (%CV)| 12.0     | 7.3       | 8.8       |
| Analytical variation (%CV)| 5.0      | 5.3       | 7.0       |
| Total variation (%CV)     | 13.0     | 8.8       | 11.2      |
| Critical difference (%)   | 36.0     | 24.4      | 31.0      |

CV=Coefficient of variation
F/T=Free-to-total PSA ratio

Figure 4
Sampling Issues with PSA Density

Note that the cancer in the larger prostate (with a lower PSA density) is more likely to be missed. Adapted from CA Cancer J Clin 1995;45:155.
States Life Table actuarial figures, there is a significant increase in the population longevity if 4.0 ng/ml is used as the PSA cutoff for all men compared with the use of age-adjusted cutoffs. We have observed similar results using more sophisticated statistical techniques.45

Optimal PSA Cutoff?

The optimal PSA cutoff for initiating a diagnostic work-up remains controversial. Aus et al reported on a Swedish screening study in which the researchers performed digital rectal examination, transrectal ultrasonography, and sextant biopsy in men with PSA levels greater than 3.0 ng/ml.46 They observed that among 243 men with PSA results between 3.0 and 4.0 ng/ml, 32 (13.2%) were found to have carcinoma. These additional cases represented 23% of all cancers detected.

In our own experience, confirmed by many others, despite early enthusiasm for PSA velocity, PSA density, and age-specific PSA cutoffs, the performance of these “enhancements” may not be reproducible. Indeed, most experts have abandoned such adjustments and utilize a consistent cutoff for all ages.45, 47

Free Versus Complexed PSA

The most important advance in the enhancement of PSA performance would appear to be based on the discovery that PSA circulates in several molecular forms. In the ejaculate, PSA exists in the free form (i.e., non-complexed to other protein moieties). In the systemic circula-

| Author        | Biopsy | Number of Patients | PSA (ng/ml)* | Prostate Volume (cc)* | PSA Density* |
|---------------|--------|--------------------|--------------|-----------------------|-------------|
| Benson et al  | Positive 98 | 7.0 (1.7)† | 28.9 (14.6)† | 0.30 (0.15)† |
|               | Negative 191 | 6.8 (1.8) | 40.1 (20.2) | 0.21 (0.11) |
| Seaman et al  | Positive 115 | 6.87 (1.70) | 29.2 (14.2)† | 0.285 (0.147)† |
|               | Negative 311 | 6.77 (1.71) | 42.2 (21.8) | 0.199 (0.108) |
| Brawer et al  | Positive 68 | 10.7 (11.4)† | 40.5 (16.6) | 0.29 (0.41) |
|               | Negative 159 | 5.2 (5.0) | 42.6 (25.6) | 0.14 (0.14) |
| Bazinet et al | Positive 217 | 21.4 (29.6)† | 37.6 (21.4)† | 0.63 (0.86)† |
|               | Negative 317 | 9.1 (8.1) | 51.6 (27.3) | 0.21 (0.25) |
| Rommel et al  | Positive 612 | 15.5 (21.6)† | 42.7 (27.2)† | 0.47 (0.11)† |
|               | Negative 1,394 | 4.9 (4.7) | 47.0 (31.6) | 0.105 (0.09) |
| Mettlin et al | Positive 171 | 12.0 (16.0)† | 38.9 (16.4) | 0.35 (0.5)† |
|               | Negative 650 | 2.1 (2.3) | 33.5 (14.2) | 0.08 (0.09) |
| Ohori et al   | Positive 110 | 9.3 (0.3-1,320)‡ | 28.1 (15.1-228.7)‡ | 0.21 (0.009-39.3)‡ |
|               | Negative 134 | 4.8 (0.2-64.1)‡ | 40.1 (13.3-332.6)‡ | 0.09 (0.007-1.82)‡ |

* Data reported as mean (standard deviation).
† p < 0.05
‡ Data reported as median (range), p < 0.05
Figure 5
Prostate Cancer and Gland Volume (N=2,135)

Positive predictive value of finding cancer of the prostate on transrectal ultrasound and prostate needle biopsy, relative to prostate gland volume.

Figure 6
Comparison of PSA, PSA Density, and Transition Zone PSA Density Curves

Receiver-operator characteristic curves comparing PSA, PSA density (PSAD), and transition zone PSA density (PSATZD) in the total PSA range. (AUC = Area Under Curve) (CAP = carcinoma of the prostate).
tion, however, PSA is complexed to a number of protease inhibitors.

Alpha-1 antichymotrypsin and alpha-2 macroglobulin are the most prevalent complexes present in serum. When PSA is complexed with alpha-1 antichymotrypsin, two epitopes remain unmasked—this complex can be detected with immunoassays. In contrast, when PSA is complexed with alpha-2 macroglobulin, all epitopes are sterically hindered, thus making this moiety undetectable by currently available assays.

Scandinavian groups led by Stenman and Lilja were pioneers in elucidating the nature of the circulating molecular forms of PSA. Stenman and associates demonstrated that the free form of PSA exists in a higher proportion in those men without prostate cancer than in those with the disease, although the majority of circulating PSA is complexed to the alpha-1 antichymotrypsin.

Christennson et al further capitalized on this observation by realizing that they could significantly enhance the specificity of the test by calculating the ratio of free-to-total PSA, or the free-to-complexed PSA as compared with total PSA alone. These findings have been confirmed by numerous other investigators, and multiple aspects of percent free PSA have recently been reviewed by Woodrum et al.

The definitive study on the performance of the free-to-total PSA was recently reported by Catalona et al. In a multicenter trial involving seven institutions, the Hybritech free and total PSA were evaluated in men who had total PSA levels between 4.0 and 10.0 ng/ml and negative digital rectal examinations, and who were undergoing systematic sector ultrasound-guided prostate needle biopsy. Of 773 men...
who were evaluated, 379 (49%) were ultimately found to have cancer. As expected, the total PSA was significantly higher in men with cancer, whereas the free-to-total PSA ratio was higher in those men with negative biopsies. Utilizing sensitivity analysis with cutoffs to provide 90% and 95% sensitivity, the specificity observed in these studies was 29% and 20%, respectively. In a man with a total PSA level between 4.0 and 10.0 ng/ml, the yield for cancer may vary drastically, depending on percent of free PSA (Table 8).

**FREE-TO TOTAL PSA ASSAY**
A number of issues remain with respect to elucidating the role of calculating the free-to-total PSA ratio. These include such factors as identifying a target population in which to measure this analyte (i.e., all men versus patients with PSA levels in a “diagnostic gray zone,” those with total PSA results between 4.0 and 10.0 ng/ml, patients with a negative biopsy, etc.)

A number of analytical problems also represent significant concerns, such as the fact that PSA assays developed by different manufacturers may result in differing serum level determinations. For example, we have demonstrated that the Abbott IMx PSA repeatedly yields lower PSA values—on the same patient sera—compared with the Hybritech Tandem method. Moreover, we have shown that the Chiron PSA 2 assay results are generally equivalent to those reported by Hybritech and Company.

Differences between manufacturers would be compounded when two analytes are measured to obtain the ratio of the free-to-total PSA. In an attempt to assess the magnitude of this effect, we compared three different manufacturers’ free PSA (Hybritech Tandem-R, Dianon Systems, and Chiron ACS-180) and utilized the Hybritech Tandem-R total PSA for all (Table 9). At 90%, 95%, and 100% sensitivity, for example, the specificity achieved with the Hybritech and Chiron methods was essentially equivalent, but lower performance was observed with the Dianon assay.

In a more recent investigation, we compared free assays and total PSA assays by three different manufacturers. This investigation also confirmed substantial equivalence between the methods developed by Hybritech and Chiron, whereas discrepancies were discovered in results reported with the Boehringer tests (Table 10).

These data clearly demonstrate the potential for problems of interpretation in determining the free-to-total PSA ratio. Laboratories must provide information with respect to which free and total assays they utilize, as well as provide meaningful risk assessment for malignancy at a given ratio. Clinicians should not apply published reports of cancer risk assessment for a given free/total cutoff to

### Table 7

| Age-specific PSA level** | PSA 4.0 ng/ml |
|--------------------------|--------------|
| PSA Level Exceeds Cutoff Point | 8.2% | 14.8% |
| Positive Predictive Value | 42% | 37% |
| Cancer Detection Rate | 3.8% | 5.7% |
| Life Saved | 757 years | 1,091 years |

* Data from Etzioni
** Cutoffs from Oesterling et al
their own patients if different assays are employed by their labs.

The discovery that PSA complexed to alpha-1 antichymotrypsin occurs in a greater proportion of men with malignancies prompted a number of investigators to attempt to develop immunoassays that would recognize this form of PSA. As immunoassays were more readily generated against the free form of PSA, however, the ratio of the free-to-total PSA was substituted as a measure of the complexed portion.

Recently, the Bayer Corporation successfully developed an assay that is specific for complexed PSA.58 We have carried out a preliminary investigation with this assay and compared it with the Hybritech total and Hybritech free-to-total assays.59 We measured total, complexed, and free PSA in 300 men, 75 with biopsy-proven carcinoma and 225 who had undergone systematic sector biopsy with benign histology (Fig. 8) As is readily apparent, there is a general trend toward enhanced specificity with the complexed PSA relative to the total PSA at similar sensitivities. Moreover, this specificity enhancement seems to be greater than that afforded by the free-to-total PSA ratio.

These findings, the implications of which are far ranging, have been recently confirmed by Sokoll and associates.60 It would appear that a single measurement of complexed PSA will perform with essentially the same sensitivity of total PSA while providing enhanced specificity. This not only has economic advantages, but also represents methodologic simplicity relative to the need to measure both the free and total PSA analytes.

Despite the impressive performances of PSA alone, its derivatives, and different forms, significant efforts are underway to investigate novel prostatic markers.

**Prostate-Specific Membrane Antigen (PSMA)**

Prostate-specific membrane antigen, first described by Horoszewicz,61 may represent an excellent addition to our prostate tumor marker armamentarium. PSMA is a 750 amino-acid Type-2 transmembrane glycoprotein consisting of three domains: An intracellular, a transmembrane re-

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**Table 8**

| PSA        | Probability of Cancer |
|------------|-----------------------|
| 2 ng/ml    | 1%                    |
| 2-4 ng/ml  | 15%                   |
| 4-10 ng/ml | 25%                   |
| > 10 ng/ml | > 50%                 |

| % FPSA   | Probability of Cancer |
|----------|-----------------------|
| 0-10%    | 56%                   |
| 10-45%   | 28%                   |
| 15-20%   | 20%                   |
| 20-25%   | 16%                   |
| > 25%    | 8%                    |

Percent Free PSA (% FPSA) can be used to further stratify risk for men with PSA values between 4 and 10 ng/ml. DRE=Digital rectal exam
gion, and a large extracellular sequence making up the bulk of the molecule.

PSMA is a distinct protein from PSA. It has been shown to be an effective marker for prostatic epithelium and appears, unlike PSA, to be more frequently expressed in more aggressive cancers. For example, Bostwick et al demonstrated that whereas 70% of benign epithelium expresses the marker, it was demonstrated in 78% of the proliferative cells that make up prostatic intraepithelial neoplasia and 80% of invasive prostate cancer cells. PSMA has been

| Table 9                      | Comparison of Manufacturer Differences Among Free PSA Assays |
|------------------------------|-------------------------------------------------------------|
|                              | Hybritech Free PSA   | Dianon Free PSA   | Chiron Free PSA |
|                              | Hybritech Total PSA | Hybritech Total PSA | Hybritech Total PSA |
| Sensitivity (%)  | % F/T Cutoff | Specificity | % F/T Cutoff | Specificity | % F/T Cutoff | Specificity |
| 90                  | 20           | 44            | 33           | 22            | 34           | 33            |
| 95                  | 22           | 38            | 34           | 19            | 35           | 32            |
| 100                 | 29           | 16            | 42           | 13            | 43           | 16            |

% F/T=percent free/total PSA

Diagnostic performance at set levels of sensitivity among men in the 2-to-20-ng/ml range (25 men with cancer, 100 men with no evidence of malignancy).

| Table 10                      | Comparison of Sensitivity and Specificity of Total PSA and Free-to-Total PSA Ratio |
|------------------------------|----------------------------------------------------------------------------------|
| Assay                        | Sensitivity (%) | Specificity (%) | Cutoff Total PSA (ng/ml) | Cutoff F/T PSA (%) |
| ACS: 180 PSA2 and Free PSA   | 100            | 0              | 0.02                     | 90                 |
| Enzymun PSA and Free PSA     | 95             | 10             | 1.7                      | 25                 |
| Tandem-R PSA and Free PSA    | 90             | 24             | 3.3                      | 15                 |

F/T= free/total PSA

Compares the sensitivity and specificity of total PSA and the free-to-total PSA ratio. Data from all patients in the benign prostatic hypertrophy and prostate cancer groups are included.
used in a number of settings, including immunoscintigraphy. This antigen forms the cornerstone, for instance, of the ProstaScint Imaging Scan (Cytogen Corporation, Princeton, NJ) used to detect occult metastatic disease.

Since the pioneering report by Horoszewicz, a number of assays for PSMA are being developed, including Western block-based assays and more recently, immunoassays. These studies have demonstrated increased levels of PSMA in patients with prostatic carcinoma. Moreover, in a series of patients who had undergone radical prostatectomy and were subsequently followed, there was a trend toward rising PSMA levels in those patients whose disease showed clinical progression and a fall in those in remission. Finally a number of investigators have utilized primers based on PSMA and reverse transcriptase polymerase chain reaction to detect circulating prostate cancer cells.

In addition to its diagnostic and staging potential, PSMA represents the basis of a novel immunotherapeutic approach for advanced prostate cancer. A number of trials, including a current phase II study, are underway in which dendritic cells are primed with PSMA antigen and reinfused into the patient in an attempt to generate an immunospecific response. We anticipate that PSMA will be an important diagnostic and therapeutic marker for future trials.

hK2

Human kallikrein 2 (hK2) is another serum protease that has approximately 80% sequence homology with PSA. It was recently discovered that hK2 acts to cleave so-called pro-PSA into its active form and that hK2 stains immunohistochemical preparations of virtually all prostatic carcinoma. Unlike PSA, but similar to PSMA, there is increased intensity of staining as is seen with the progression from benign prostatic epithelium to high-grade prostatic intraepithelium neoplasia, and to carcinoma. Serum assays for hK2 are under development, although cross-reactivity with other serum proteins may limit their utility. If reliable assays are developed, however, this, too, may become an important marker for prostate cancer in the future.

Conclusion

PSA has revolutionized the management of men with prostate cancer. Its role in
screening and early detection is both well founded and unprecedented. Undoubtedly, new approaches to interpreting PSA levels in the individual patient will be discovered, and new markers will be identified to aid clinicians in caring for men with prostate cancer, the most common human neoplasm.

References
1. Landis SH, Murray T, Bolden S, Wingo PA: Cancer Statistics, 1999. CA Cancer J Clin 1999; 49:8-31.
2. Brawer MK, Kirby RS: Fast Facts: PSA. London, Health Press: 1998
3. Chapters, from p 389-437, in Oesterling JE, ed. The Urologic Clinics of North America, Vol 24. Philadelphia: WB Saunders; 1997.
4. Nixon RG, Brawer MK: Refinements in serum prostate-specific antigen testing for the diagnosis of prostate cancer in Kirby RS, O’Leary MP (eds): Recent Advances in Urology. Edinburgh, U.K., Churchill Livingstone, 1998.
5. Wang MC, Valenzuela LA, Murphy GP, Chu TM: Purification of a human prostate specific antigen. Invest Urol 1979;17:159-163.
6. Stamey TA, Yang N, Hay AR, et al: Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 1987;317:909-916.
7. Ercole CJ, Lange PH, Mathisen M, et al: Prostate specific antigen and prostatic acid phosphatase in the monitoring and staging of patients with prostatic cancer. J Urol 1987;138:1181-1184.
8. Ferro MA, Barnes I, Roberts JB, Smith PJ: Tumor markers in prostatic carcinoma: A comparison of prostate-specific antigen with acid phosphatase. Br J Urol 1987;60:69-73.
9. Hudson MA, Bahnson RR, Catalona WJ: Clinical use of prostate specific antigen in patients with prostate cancer. J Urol 1989;142:1011-1017.
10. Brawer MK, Rennels MA, Nagle RB, et al: Serum prostate specific antigen and prostate pathology in men having simple prostatectomy. Am J Clin Pathol 1989;92:760-764.
11. Irani J, Levillain P, Goujon JM, et al: Inflammation in benign prostatic hyperplasia: Correlation with prostate specific antigen value. J Urol 1997;175:1301-1303.
12. Neal DE Jr, Clejan S, Sarma D, Moon TD: Prostate specific antigen and prostatitis. I: effect of prostatitis on serum PSA in the human and non human primate. Prostate 1992;20:105-111.
13. Cooner WH, Mosley BR, Rutherford CL Jr, et al: Prostate cancer detection in a clinical urological practice by ultrasonography, digital rectal examination and prostate specific antigen. J Urol 1990;143:1146-1154.
14. Brawer MK, Lange PH: PSA in the screening, staging and follow up of early-stage prostate cancer: A review of recent developments. World J Urol 1989;7:7-11.
15. Hodge KK, McNeal JE, Terris MK, Stamey TA: Random systematic versus directed ultrasound guided transrectal core biopsies of the prostate. J Urol 1989;142:71-75.
16. Catalona WJ, Smith DS, Ratlifl TL, et al: Measurement of prostate specific antigen in serum as a screening test for prostate cancer. N Engl J Med 1991;324:1156-1161.
17. Brawer MK, Chetner MP, Beatie J, et al: Screening for prostatic carcinoma with prostate specific antigen. J Urol 1992;147:841-845.
18. Babaian RJ, Camps JL: The role of prostate specific antigen as part of the diagnostic triad and as a guide when to perform a biopsy. Cancer 1991;68:2060-2063.
19. Bazinet M, Meshref AW, Trudel C, et al: Prospective evaluation of prostate-specific antigen density and systematic biopsies for early detection of prostatic carcinoma. Urology 1994;43:44-51.
20. Catalona WJ, Richie JP, Ahmann FR, et al: Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: Results of a multicenter clinical trial of 6,630 men. J Urol 1994;151:1283-1290.
21. Cooner WH, Mosley BR, Rutherford CL Jr, et al: Clinical application of transrectal ultrasonography and prostate specific antigen in the search for prostate cancer. J Urol 1988;139:758-761.
22. Ellis WJ, Chetner MP Preston SD, Brawer MK: Diagnosis of prostatic carcinoma: The yield of serum prostate specific antigen, digital rectal examination and transrectal ultrasonography. J Urol 1994;152:1520-1525.
23. Mettlin C, Littrup PJ, Kane RA, et al: Relative sensitivity and specificity of serum prostate specific antigen (PSA) level compared with age-referenced PSA, PSA density and PSA change: Data from the American Cancer Society National Prostate Cancer Detection Project. Cancer 1994;74:1615-1620.
24. Rommel FM, Agusta VE, Breslin JA, et al: The use of prostate specific antigen and prostate specific antigen density in the diagnosis of prostate cancer in a community based urology practice. J Urol 1994;151:88-93.
25. Moon TD, Clejan S, Neal DE Jr: Prostate-specific antigen and prostatitis. II: PSA production and release kinetics in vitro. Prostate 1992;20:113-116.
26. Mettlin C, Jones G, Averette H, al el: Defining and updating the American Cancer Society guidelines for the cancer-related checkup: Prostate and endometrial cancers. Cancer 1993;43:42-46.
27. AUA: American Urological Association policy statement: Early detection of prostate cancer and use of transrectal ultrasound. Baltimore, 1992.
28. Brawer MK: How to use prostate specific antigen in the early detection or screening for prostatic carcinoma. CA Cancer J Clin 1995;45:148-164.
29. Williford WO, Lepor H, Dixon CM, et al: Serum PSA levels after 52 weeks of therapy with finasteride, terazosin, combination, and placebo: Results of the VA Cooperative study #359. J Urol (Suppl) Vol 155;1996;533A.
30. Carter HB, Morrell CH, Pearson JD, et al: Estimation of prostatic growth using serial prostate specific antigen measurements in men with and without prostate disease. Cancer 1992;52:3323-3328.
31. Porter JR, Hayward R, Brawer MK: The significance of short-term PSA change in men undergoing ultrasound guided prostate biopsy. J Urol 1994;264:151:5:293A.
32. Nixon RG, Wener MH, Smith KM, et al: Biological variation of prostate specific antigen levels in serum: An evaluation of day-to-day physiological fluctuations in a well-defined cohort of 24 patients. J Urol 1997;157:2183-2190.
33. Nixon RG, Wener MH, Smith KM, et al: Day to day changes in free and total PSA: Significance of biological variation. PCPD 1998;1:90-96.
34. Babaian RJ, Fritsche HA, Evans RB: Prostate specific antigen and prostate gland volume: Correlation and clinical application. J Clin Lab Anal 1990;4:135-137.
35. Benson MC, Whang IS, Olsson CA, et al: The use of prostate specific antigen density to enhance the predictive value of intermediate levels of serum prostate specific antigen. J Urol 1992;147:817-821.
36. Brawer MK, Aramburu EA, Chen GL, et al: The inability of prostate specific antigen index to enhance the predictive value of PSA in the diagnosis of prostatic carcinoma. J Urol 1993;150:369-373.
37. Seaman E, Wanger M, Olsson CA, et al: PSA density (PSAD): Role in patient evaluation and management. Urol Clin North Am 1993;20:653-663.
38. Ohori M, Dunn JK, Scardino PT: Is prostate-specific antigen density more useful than prostate-specific antigen levels in the diagnosis of prostate cancer? Urology 1995;46:666-671.
39. Lin DW, Gold MH, Ransom S, et al: Transition zone prostate specific antigen density: Lack of use in prediction of prostatic carcinoma. J Urol 1998;160:77-82.
40. Kalish J, Cooner WH, Graham SD Jr: Serum PSA adjusted for volume of transition zone (PSAT) is more accurate than PSA adjusted for total gland volume (PSAD) in detecting adenocarcinoma of the prostate. Urology 1994;43:601-606.
41. Maeda H, Ishiota S, Maekawa S, et al: Prostate specific antigen density in the transition zone in the detection of prostate cancer. J Urol 1997; Vol. 2193. 158:4:58A.
42. Zlotta AR, Djavan B, Marberger M, Schulman CC: Prostate specific antigen density of the transition zone: A new effective parameter for prostate cancer prediction. J Urol 1997;157:1315-1321.
43. Oesterle JE, Jacobsen SJ, Chute CG, et al: Serum prostate specific antigen in a community-based population of healthy men: Establishment of age-specific reference ranges. JAMA 1993; 270:860-864.
44. Dalkin BL, Ahmann FR, Kopp JB: Prostate specific antigen levels in men older than 50 years without clinical evidence of prostatic carcinoma. J Urol 1993;150:1837-1839.
45. Etzioni R, Shen Y, Petteway JC, Brawer MK: Age-specific prostate specific antigen: A reassessment. Prostate 1996;7:70-77.
46. Aus G. (Eighth International Prostate Cancer Update). Screening for Prostate Cancer in Sweden. February 1998.
47. Borer JG, Serman J, Solomon MC, et al: Age-specific reference ranges for prostate-specific antigen and digital rectal examination may not safely eliminate further diagnostic procedures. In: Urol J, ed. 155:48A;1996:48A.
48. Stenman UH, Leinonen J, Althman H, et al: A complex between PSA and a 1-antichymotrypsin is the major form of prostate specific antigen in serum of patients with prostatic cancer: Assay of the complex improves clinical sensitivity for cancer. Cancer Res 1991;51:222-226.
49. Lilja H, Christensson A, Dahlun E, et al: Prostate specific antigen in serum occurs predominantly in complex with alpha-1 antichymotrypsin. Clin Chem 1991;37:1618-1625.
50. Christensson A, Bjork T, Nilsson O, et al: Serum prostate specific antigen complexed to alpha 1-antichymotrypsin as an indicator of prostate cancer. J Urol 1993;150:100-105.
51. Luderer AA, Chen Y, Soriano TF, et al: Measurement of the proportion of free to total prostate specific antigen improves diagnostic performance of prostate specific antigen in the diagnostic gray zone of total prostate specific antigen. Urology 1995;46:187-194.
52. Woodrum DL, Brawer MK, Partin AW, et al: Interpretation of free prostate specific antigen clinical research studies for the detection of prostate cancer. J Urol 1998;159:5-12.
53. Catalona WJ, Partin AW, Slawin KM, et al: Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: A prospective multicenter clinical trial. JAMA 1998;279:1542-1547.
54. Brawer MK, Daam P, Petteway JC, Wener MH: Assay variability in serum prostate specific antigen determination. Prostate 1995;26:3-6.
55. Brawer MK, Bankson DD, Haver VM,
Petteway JC: Comparison of three commercial PSA assays: Results of Restandardization of the Ciba Corning method. Prostate 1997;30:269-273.
56. Nixon RG, Meyer GE, Blase AB, et al: Comparison of 3 investigational assays for the free form of prostate specific antigen. J Urol 1998;160:420-425.
57. Roth HJ, Christensen-Stewart S, Brawer MK: A comparison of three free and total PSA assays. PCPD 1998;1:326-331.
58. Allard WJ, Zhou Z, Yeung KK: Novel immunoassay for the measurement of complexed prostate-specific antigen in serum. Clin Chem 1998;44:1216-1223.
59. Bostwick DG, Pacelli A, Blute M, et al: Measurement of complexed PSA improves specificity for early detection of prostate cancer. Urology 1998;52:372-378.
60. Sokoll LJ, Bruzek DJ, Cox JL, et al: Is complexed PSA alone clinically useful? J Urol (Suppl) 1998; Vol. 159; 1998:234A.
61. Horoszewicz JS, Kawinski E, Murphy GP: Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients. Anticancer Res 1987;7:927-935.
62. Bostwick DG, Pacelli A, Blute M, et al: Prostate specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinoma: A study of 184 cases. Cancer 1998;82:2256-2261.
63. Babaian RJ, Sayer J, Podoloff DA, et al: Radioimmunoscintigraphy of pelvic lymph nodes with 111Indium-labeled monoclonal antibody cyt-356. J Urol 1994;152:1952-1955.
64. Kahn D, Williams RD, Seldin DW, et al: Radioimmunoscintigraphy with 111Indium labeled CYT-356 for the detection of occult prostate cancer recurrence. J Urol 1994;152:1490-1495.
65. Haseman MK, Reed NL, Rosenthal SA: Monoclonal antibody imaging of occult prostate cancer in patients with elevated prostate-specific antigen: Positron emission tomography and biopsy correlation. Clin Nucl Med 1996;21:703-713.
66. Kahn D, Haseman M, Libertino J, et al: Indium-111 capromab pendetide (ProstaScint) imaging of patients with rising PSA post-prostatectomy. J Urol 1997;Vol. 157 (Suppl):204A.
67. Troyer JK, Beckett ML, Wright GL Jr: Detection and characterization of the prostate-specific membrane antigen (PSM) in tissue extracts and body fluids. Int J Cancer 1995;62:552-558.
68. Rochon YP, Horoszewicz JS, Boynton AL, et al: Western blot assay for prostate-specific membrane antigen in serum of prostate cancer patients. Prostate 1994;25:219-223.
69. Holmes EH, Greene TG, Tino WT, et al: Analysis of glycosylation of prostate-specific membrane antigen derived from LNCaP cells, prostatic carcinoma tumors, and serum from prostate cancer patients. Prostate Suppl 1996;7:25-29.
70. Murphy G, Ragde H, Kenny G, et al: Comparison of prostate specific membrane antigen, and prostate-specific antigen levels in prostatic cancer patients. Anticancer Res 1995;15:1473-1479.
71. Murphy GP, Tino WT, Holmes EH, et al: Measurement of prostate-specific membrane antigen in the serum with a new antibody. Prostate 1996;28:266-271.
72. Israeli RS, Miller WH Jr, Su SL, et al: Sensitive nested reverse transcription polymerase chain reaction detection of circulating prostatic tumor cells: Comparison of prostate-specific membrane antigen and prostate-specific antigen-based assays. Cancer Res 1994;54:6306-6310.
73. Lintula S, Stenman UH: The expression of prostate-specific membrane antigen in peripheral blood leukocytes. J Urol 1997;157:1969-1972.
74. Israeli RS, Miller WH Jr, Su SL, et al: Sensitive detection of prostatic hematogenous tumor cell dissemination using prostate specific antigen and prostate specific membrane-derived primers in the polymerase chain reaction. J Urol 1995;153:573-577.
75. Price DK, Woodard WL, Teigland CM: Simultaneous detection of circulating prostate-specific antigen (PSA)-expressing and prostate-specific membrane antigen (PSMA)-expressing cells by a multiplex reverse transcriptase polymerase chain reaction (RT-PCR) assay. Urol Oncol 1995;1:226-233.
76. Loric S, Dumas F, Eschwege P, et al: Enhanced detection of hematogenous circulating prostatic cells in patients with prostate adenocarcinoma by using nested reverse transcription polymerase chain reaction assay based on prostate-specific membrane antigen. Clin Chem 1995;41:1698-1704.
77. Cama C, Olsson CA, Raffo AJ, et al: Molecular staging of prostate cancer. II: A comparison of the application of an enhanced reverse transcriptase polymerase chain reaction assay for prostate specific antigen versus prostate specific membrane antigen. J Urol 1995;153:1373-1378.
78. Sokoloff MH, Tso CL, Kaboo R, et al: Quantitative polymerase chain reaction does not improve preoperative prostate cancer staging: A clinicopathological molecular analysis of 121 patients. J Urol 1996;156:1560-1566.
79. Darson MF, Pacelli A, Roche P, et al: Human glandular kallikrein 2 (hK2) expression in prostatic intraepithelial neoplasia and adenocarcinoma: A novel prostate cancer marker. Urology 1997;49:857-862.