Monitoring Percent Free PSA in Serial Specimens: Improvement of Test Specificity, Early Detection, and Identification of Occult Tumors

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We have measured the serum concentration of prostate specific antigen (tPSA) and determined the percent free PSA (% fPSA) in serial specimens from 64 patients with prostate cancer, 35 patients with benign prostate hyperplasia (BPH), and 3 patients with prostatitis. We found that the % fPSAs were not a constant for individual patients during the course of the disease. When we compared only the % fPSA of the first specimen of serial specimens from individual patients, who were largely untreated, 37% of BPH specimens were above 22%, whereas only 1.6% cancer samples were above that value. We also found that 67% of cancer specimens and 14% of BPH samples were below 8%, respectively. Although % fPSA distribution pattern remained similar be-

tween two types of specimens, less differentiation was found between BPH and prostate cancer in random specimens compared to the study using first specimens of an individual patient's serial samples. Percent fPSA apparently are affected by treatment. However, the most important benefit for the determination of % fPSA appears to be the sensitivity of % fPSA to identify occult tumors when the tPSAs were in the normal concentration range. Determination of % fPSA also seems to improve the specificity of tPSA, not only during screening for the differentiation between BPH and prostate cancer, but also during monitoring of treatment and recurrence. *J. Clin. Lab. Anal.* 12:26–31, 1998. © 1998 Wiley-Liss, Inc.

Key words: BPH; benign prostate hyperplasia; prostate cancer

INTRODUCTION: AVOIDING UNNECESSARY BIOPSIES

Prostate specific antigen (PSA) is a serine protease and a member of the kalikrane family. Because of the presence of many protease inhibitors in the serum, PSA does not exist in the serum entirely as free molecules, but binds to various protease inhibitors in complex forms (1–3). Immunologically, only two major forms can be detected in the serum: free PSA (fPSA) and PSA- α 1-antichymotrypsin (PSA-ACT) complex (4,5). The percent distribution of these two forms of PSA in serum is not a fixed constant. In general, fPSA is found at ~0–5% in patients with prostate cancer (6), whereas a much higher percentage of fPSA is usually found in patients with benign prostate hyperplasia (BPH) and prostatitis.

Because of its relative tissue specificity, PSA was the first tumor marker recommended, in combination with digital rectal examination, for screening of prostate cancer (7). Screening should be undertaken annually for men > 50 years old, since it is important to detect any highly curable, organ-confined

tumors before they become metastasized (3). When the tumor is still localized, the serum PSA level usually falls between 4–10 ng/mL. Unfortunately, PSA is not specific for prostate cancer; a large percentage of patients with BPH also exhibit serum PSA in this concentration range. In the past, biopsies were needed to confirm the diagnosis. Consequently, BPH patients were also subjected to this costly and painful procedure. Recently, it was suggested that the determination of percent fPSA may help differentiate BPH from prostate cancer, thus reducing many unnecessary biopsies.

Many different cutoff values for % fPSA have been established by various investigators in order to identify BPH patients during screening to avoid unnecessary biopsies when the tPSA values fall between 4–10, or 3–10 ng/mL. These cutoff values are similar to the 20% earlier proposed by

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Received 6 June 1997; Accepted 12 June 1997

Catalona et al. (8). Their formulation depends upon what percent of BPH one wants to identify and how many prostate cancers one is willing to leave possibly undetected. There is no doubt that this approach will improve the specificity of the PSA test for screening. An excellent recent review by Vashi and Oesterling covers this aspect in detail (9).

It is well known that 38–48% of patients with clinically significant organ-confined cancer have a normal tPSA value of 4 ng/mL or less (10). Using a lower limit for tPSA during screening, perhaps 2.5 or 3.0 ng/mL, will result in an increased rate of cancer detection (9). Based on their ROC curve (receiver operating characteristic curve), Vashi et al. (9) concluded that in the concentration range of 3.0–4.0 ng/mL, % fPSA is much better than the tPSA for distinguishing prostate cancer from benign prostate disease. The study by Catalona et al. (11) also supports the ability of % fPSA to improve cancer detection when the total PSA value is in the normal range. In a prospective study, using 25% as a cutoff value for % fPSA, Catalona et al. (11) detected 91% of the cancer in “screened volunteers” whose tPSA concentrations fell between 2.6–4.0 ng/mL, and 26% of the negative biopsies were eliminated.

In the present study we have monitored the serum PSA (tPSA) and determined the % fPSA in serial specimens. We calculated the % fPSA by dividing the individual value of fPSA by the sum of PSA-ACT complex plus fPSA, not by the directly measured tPSA concentrations. We found that the value of % fPSA in serial specimens fluctuated during the course of the disease. We also discovered that the determination of % fPSA aided not only in the differentiation of BPH from prostate cancer during screening, but also helped identify occult tumors when the tPSA concentrations fell within the normal range. We believe that the measurement of fPSA and the calculation of % fPSA also will improve the specificity of tPSA assay during treatment and during monitoring recurrence.

MATERIALS AND METHODS

To establish various in-house PSA assays, we purchased polyclonal anti-PSA antibody from Dako Corp. (Carpinteria, CA) and monoclonal anti-fPSA and enzyme conjugated anti-ACT from CanAg Diagnostics (Gothenburg, Sweden) and BioDesign (Camel, NY). For miscellaneous chemicals, we obtained BSA (Bovine serum albumin, fraction V), Tween 20, casein, and thimerosal from Sigma (St. Louis, MO). The microtiter Strips (immulon 4) was from Dynatech Laboratories (Chantilly, VA). Sheep antirabbit IgG HRP (horseradish peroxidase) conjugate solution was obtained from BioDesign International (Kennebunk, ME). The TMB substrate kit was from Pierce (Rockford, IL).

Clinical Specimens

All specimens were obtained from Department of Urology, School of Medicine at the University of Utah Health

Complex Center. The total PSA was determined on fresh specimens on IMx instrument using the Abbot PSA kit. The specimens were kept frozen and the fPSA and PSA-ACT were measured at the same time a few months or a few years later. The majority of the specimens were obtained from patients with diagnoses confirmed by biopsies.

PSA-ACT and fPSA Assay

Both fPSA and PSA-ACT assays were developed in house on the microplate following the sandwich format (9). Polyclonal anti-PSA antibody was used in both assays to coat the microwell to trap the analytes. The trapped fPSA was detected by monoclonal anti-fPSA antibody and the captured PSA-ACT complex was quantified by polyclonal anti-ACT antibody. Our PSA-ACT assay is surprisingly specific with very low cross-reactivity with either fPSA and ACT (12). Our fPSA assay can detect as low as 0.06 ng of fPSA per mL and the cross-reactivity with PSA-ACT is only 0.27%, which enables us accurately to measure serum fPSA in the presence of much higher serum concentrations of PSA-ACT complex. Our PSA-ACT assay also has very low cross-reactivity with ACT, so that the excess amount of ACT present in the serum does not affect the measurement of serum the PSA-ACT complex.

Percent fPSA

The % fPSA was calculated by dividing the value of fPSA by the sum of fPSA and PSA-ACT complex.

RESULTS

At the present time, all PSA assays measure both free PSA (fPSA) and PSA-ACT in the serum and are therefore designated as total PSA (tPSA) assay. Although other PSA complexes, such as PSA- α_2 -macroglobuline, PSA- α_1 -antitrypsin, and PSA-inter- α -trypsin also may exist, they do so either in negligible amounts or are not detectable immunologically (5,9). Practically speaking, free PSA and the PSA-ACT complex together account for the majority of PSA isoforms quantified by the current tPSA assay in the serum. A new generation of assays has emerged lately. They are assays either specific for fPSA or for the PSA-ACT complex. In this study the tPSA of the fresh serial specimens were measured using the Abbott PSA kit on IMx instrument. However, both the serum concentrations of free PSA and PSA-ACT complex were measured on frozen specimens (kept frozen at -70°C from a few months to a few years) with our in-house kits (12). Measured fPSA was expressed as % fPSA, which is fPSA over the sum of fPSA plus PSA-ACT complex, then multiplied by 100. We divided fPSA by (fPSA + PSA-ACT), not tPSA, because we wanted to confine ourselves to exactly what was measured. Regardless of the assay kit used for tPSA measurement, in our experience, the value of tPSA always exceeded the sum of fPSA plus PSA-ACT, and the difference between the total PSA and the sum of fPSA and

PSA-ACT complex varied from specimen to specimen. Sometimes the difference was only a few percent of the total, sometimes up to 20% or 30%. Reasons for this variation were not clear. Since even at the present time all commercial PSA kits do not agree with each other (13,14), we believe that expressing fPSA over the sum of fPSA and PSA-ACT makes the value of % fPSA more precise and more interpretable.

Fluctuation of % fPSA Values

Monitoring serial specimens from individual patients revealed that the % fPSA is not a constant value for each patient. The values of % fPSA obtained from the serial specimens of the same patient will undergo changes over time during treatment. This type of fluctuation could be detected in every type of clinical situation. As illustrated in Figure 1, fluctuations of % fPSA during the course of the disease could change from > 20%, favoring benign disease, to < 10%, favoring prostate cancer. The % fPSA appears to be a sensitive marker for monitoring treatment. Understandably, one must be more cautious when correlating the value of % fPSA with either prostate cancer or BPH without full knowledge of the exact clinical status of the patient, including gland volume, tumor activity, and type of treatment. There is much for us to learn about the alteration of % fPSA and its relationship with specific changes of clinical situations. At the present time, determination of % fPSA is more useful during screening for prostate cancer before any treatment starts.

Percent fPSA of Random and 1st Specimen of Individual Serial Samples

The value of the determination of % fPSA during screening to differentiate BPH from prostate cancer has been dem-

Fig. 1. Fluctuation of % fPSA during monitoring of the disease. This is to illustrate that the % fPSA is not a constant value for individual patient. This patient had prostate cancer and was confirmed by biopsy. No prostatectomy was ever performed. Values next to the square symbols are tPSA concentrations. The tPSA was determined by Abbott assay on IMx. % fPSA was calculated as $fPSA / (fPSA + PSA-ACT \text{ complex}) \times 100$.

onstrated repeatedly by various investigators. When the % fPSA of random specimens from BPH and prostate cancer (CAP) patients were compared, a similar pattern found with screening samples was observed (Fig. 2). The % fPSA determined in random specimens also showed a large overlap between CAP and BPH even though samples from BPH tend to have a higher percent of fPSA. Without the knowledge of gland volume, if we selected 22% as the cutoff point to differentiate between BPH and prostate cancer, without the information of gland volume, we found that 32% of the BPH specimens would be > 22%, whereas only 10% of specimens from prostate cancer patients exceeded this value (Fig. 2). In addition, 39% of cancer specimens were < 8%, whereas only 10% BPH samples were less than this cutoff point. Therefore, even during the treatment, differences in terms of % fPSA between BPH and prostate cancer can still exist. In fact, the two cutoff values, such as 22% and 8%, are not too much different from values selected by other investigators using screening of prostate cancer (8,9,11).

However, when we selected only the % fPSA of the first of the serial specimens from individual patients, which were more likely to be free of any treatment, when we compared BPH and cancer patients (Fig. 3), we found that the difference between prostate cancer and BPH became much more distinct. Applying the same cutoff values used for the random specimens, we found 37% (not 32%) of BPH specimens were above this value, whereas only 1.6% (instead of 10%) of cancer specimens exceeded 22% fPSA. Also, 67% (not 39%) of cancer specimens and only 14% of BPH samples were < 8%. Apparently, use of medications affected the % fPSA determinations and caused more overlap between BPH and prostate cancer. Nevertheless these studies also indicate that the determination of % fPSA will still provide indications of what is happening to the tumor, the prostate gland, and the patient during monitoring and treatment.

Fig. 2. Percent free PSA distribution of random specimens from patients with prostate cancer and BPH. These results from random specimens show a large overlap between prostate cancer and BPH; 22% and 8% were the cutoff points selected to differentiate BPH from prostate cancer and prostate cancer from BPH, respectively. See Figure 1 legend for other details.

Fig. 3. Percent fPSA distribution between prostate cancer and benign prostate diseases. Only the results of the first specimen of a sample series from individual patients were used for plotting. Compared to the results of random specimens (Fig. 2), there is a better distinction in % fPSA between prostate cancer and BPH. See legends, Figures 1 and 2, for other details.

Detection of Occult Tumor

We found that in addition to the possibility of aiding in the differentiation of BPH from prostate cancer, the determination of % fPSA appears to be useful as well for the detection of occult tumors. Many patients with biopsy-confirmed prostate cancer, who may not exhibit an elevation of serum tPSA, were clearly identified by their low % fPSA values. Six individual cases showing the superior specificity of % fPSA over the tPSA test are illustrated in Figure 4. The first specimen of all six patients with biopsy-confirmed prostate cancer had normal serum tPSA (equal or below 4 ng/mL), but distinctly low % fPSA values. Only the % fPSA of patient SR175 was slightly higher at 18% at the beginning, but dropped below 10% in subsequent measurements. All these cases were monitored before prostatectomy were performed. It also was surprising to find that such cases were not infrequent. In the group of 24 patients with prostate cancer, before they underwent prostatectomy, as many as six (24%) showed tPSA < 4 ng/mL, but had low % fPSA.

It also should be pointed out that most of the patients we included in this study were > 60 years of age. Therefore, if we apply the age-dependent reference range to these patients (15,16), the upper normal limit of tPSA would be > 4 ng/mL. In other words, more patients would have been considered normal based on their initial tPSA values and more patients would have been diagnosed normal based on their serum tPSA values. For this older population, % fPSA determination would have been even more reliable and their determination would have revealed (identified) more occult tumors. Moreover, Abbott values are known to be slightly higher than the tPSA values determined by the Hybritech assay. Consequently, there

Fig. 4. Identification of prostate tumors by % fPSA determinations when the tPSAs were within normal range. Cases of all patients listed in this figure did not have prostatectomy performed.

could be more patients exhibiting tPSA < 4 ng/mL while their % fPSA remained low.

Early Detection

In Figure 5, we demonstrate that there may be advantages of monitoring % fPSA during the course of a disease. All the cases shown in Figure 5 did not have prostatectomy performed. It appears that in patients with biopsy-proven prostate cancer, their samples were consistently associated with low % fPSA, even though the tPSA concentrations were not highly elevated. For example, the tPSA concentrations found in patients S3, S163, S17, and S169 were still in the range in which benign and malignant prostate diseases largely overlap. The % fPSA determinations were particularly useful in patients S35 and S118. Both cases showed decline of their tPSA, but it was the low % fPSA that confirmed the diagnosis of prostate cancer. The decline of tPSA could be a result of treatment effecting the gland volume, and the decrease of fPSA might have nothing to do with the regression of the tumor.

In contrast, patients with BPH were frequently associated with high % fPSA (Fig. 6) regardless whether their tPSA concentrations were below or above 4 ng/mL. Even though certain % fPSA values may drop close to 10% during the course of the disease, as with patient S167 and SR 268, the overall % fPSA determined would suggest that the patients were likely associated with benign prostate disease.

Similar phenomena were observed in patients after prostatectomy during monitoring of recurrence (Fig. 7). Since the

Fig. 5. Early suggestion of the presence of tumor by % fPSA before tPSA became highly elevated. All cases listed here were from patients with biopsy-confirmed prostate cancer.

detection of any level of tPSA during monitoring of recurrence is most likely associated with cancer, the % fPSA determination may not be as useful as for those without prostatectomy. Nevertheless, the specificity of tPSA measurement is improved by the % fPSA determinations.

DISCUSSION

It is well known that PSA test is not 100% sensitive for prostate cancer. Approximately one-third of patients with prostate cancer have normal serum PSA levels (17,18). When age-specific references are applied to these patients, the problem

Fig. 6. Benign prostate disease suggested by % fPSA determination. All these cases were from patients with BPH.

Fig. 7. Measurement of both tPSA and % fPSA during monitoring recurrence. The specificity of the tPSA is improved by a simultaneous determination of % fPSA.

is often worse because more older people will be considered normal even though their serum PSA levels may be > 4 ng/mL. If future studies continue to support the conclusions of our study confirming that % fPSA determination can truly improve the specificity of serum tPSA tests, we feel that the % fPSA determination should not only be performed routinely during screening and during treatment, but also during monitoring recurrence. During screening it should be determined not just for patients with serum tPSA between 4–10 ng/mL but should be extended to those with tPSA between 2.5–4 ng/mL. The value of % fPSA determination will rest not only with the capacity to differentiate between BPH and prostate cancer, but also the ability to identify occult tumors in patients with normal serum tPSA concentrations. However, when the tPSA is found to be < 2 ng/mL during screening, determination of the % fPSA will not be required, since the corresponding likelihood of cancer would be very low.

It is well known that the value of free PSA can be influenced by the volume of the prostate gland. The larger the gland, the higher will be the free PSA found in the serum. Older men have higher reference values of serum tPSA largely due to their slightly enlarged glands. Any treatment affecting the gland volume will probably have an impact on the free PSA and also the % fPSA determinations. However, the fluctuation of % fPSA observed in the present study seems to indicate that there are other factors or parameters that may also affect the % fPSA determinations. It should be remembered that any change in either fPSA or PSA-ACT concentrations will lead to a change in % fPSA value. An increase in

serum PSA-ACT concentrations is usually associated with the progression of prostate cancer, whereas an increase in fPSA has been known to be proportional to the volume of the gland. Therefore, the interpretation of % fPSA could be complicated since it represents a result of two independent variables.

Percent fPSA can be expressed either by dividing the fPSA by the serum tPSA or by the sum of serum fPSA plus serum PSA-ACT complex concentrations. Because the PSA-ACT assay is not commonly available as yet, most studies have been carried out using the serum tPSA to calculate the % fPSA values. In view of the different tPSA values produced by various commercial PSA kits, the determined % fPSA values will probably be kit dependent. It is crucial not only that one specify which of the commercial serum tPSA kits is used to calculate the % fPSA, but also that any cutoff point established with one kit not be applied to values established by another kit.

Although the majority of the serum tPSA is made up from fPSA and PSA-ACT, very seldom is the tPSA measured equal to the sum of fPSA plus PSA-ACT complex in the serum. It could be that other PSA isoforms in addition to fPSA and PSA-ACT complex exist in the serum or that current tPSA does not accurately measure the fPSA and PSA-ACT in the serum. We feel that expressing % fPSA by dividing fPSA by the sum of fPSA and PSA-ACT complex is more accurate and better defined. There are less technical problems associated with both the fPSA and PSA-ACT assays than generally with the serum tPSA assay (1).

We feel strongly that any cutoff point that we might select now for the differentiation of BPH from prostate cancer would be premature. There will be many modifications to come as we learn how to minimize or eliminate all of the factors and parameters affecting either the fPSA and PSA-ACT concentrations. The exact expression of fPSA also would impact the cutoff point we select. Any objections made to the use of % fPSA for improving the specificity of serum tPSA assay, such as by the most recent report by Murphy et al. (19), should be treated as inconclusive until all aspects of the % fPSA determination have been well defined. Presently, we still lack an answer as to why the fPSA found in BPH and prostatitis patients does not bind to ACT effectively. It is the result of different fPSA clearance rates for cancer and benign disease? Could it be related to variant ACTs that are produced by BPH and cancer patients? Or are there differences in chemical composition of fPSA from BPH and from prostate cancer? Differences could exist between proteins or carbohydrate moieties from these two different sources. Conceivably there is still plenty of latitude for improvement in the clinical utilities of the fPSA.

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