Advantages of Replacing the Total PSA Assay With the Assay for PSA- α 1-Antichymotrypsin Complex for the Screening and Management of Prostate Cancer

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Several advantages become immediately apparent when the prostate specific antigen (PSA, or tPSA) assay is replaced by the assay specific for the serum PSA- α 1-antichymotrypsin (PSA-ACT) complex. For instance, random contributions to the tPSA value by various serum minor PSA isoforms can be avoided, making possible the determination of a more accurate relation of the PSA-ACT concentration to the tumor activity. Discrepancies in percent free PSA (% fPSA) values from the same specimens due to the use of different commercial kits also can be eliminated, mainly because the PSA-ACT assay does not have the problems in antibody selection and calibrator preparation usually associated with the tPSA assay. We found that at the present time different cut-offs of % fPSA for the differentiation of BPH from prostate cancer must be established for each individual tPSA assay. Cutoffs estab-

lished using values from one tPSA assay should not be used for making clinical decisions when their tPSA values are determined by a different kit. Moreover, when we monitored the patients during treatment with serum tPSA, specific fPSA, and specific PSA-ACT complex assays simultaneously, it was clear that any interpretation of the patient's clinical status based on tPSA values alone could be misleading. Because there is less PSA-ACT complex in BPH specimens relative to that found in cancer serum samples, expressing fPSA as "fPSA/PSA-ACT $\times$ 100" and measuring PSA-ACT complex concentrations instead of tPSA during screening improve the measurable contrast between BPH and prostate cancer. Although individually modest, collectively these advantages can add up to considerable improvements. *J. Clin. Lab. Anal.* 12:32–40, 1998. © 1998 Wiley-Liss, Inc.

Key words: benign prostate hyperplasia; free PSA; percent-free PSA

INTRODUCTION: SCREENING FOR PROSTATE CANCER

Only recently did we realize that serum contains two major isoforms of prostate specific antigen (PSA), a free form and a complex form (1–3). Because PSA is a protease and because there is an abundance of protease inhibitors present in the serum, various complexes can be formed in the blood circulation between PSA and various protease inhibitors (1,4,5). However, only the PSA- α 1-antichymotrypsin (PSA-ACT) is essentially the only complex detectable immunologically (6,7). Because of the presence of two PSA isoforms, free PSA and PSA-ACT complex, in the serum, many technical problems can arise while setting up a PSA assay. (Since current PSA assays measure both fPSA and the PSA-ACT complex, we identify them as total PSA assay or tPSA assay to distinguish them from fPSA and PSA-ACT specific assays.) It was not realized during the time when these commercial kits were undergoing development that PSA assays were required to react with two different molecular forms of

PSA in the serum; a major cause of discrepancies in PSA values produced by different commercial tPSA kits. The failure to select appropriate antibodies to accommodate both free and complexed PSA molecules equally and the failure to use correct calibrator to match the composition of serum analytes largely account for many problems associated with the commercial tPSA kits (1,7).

It is well known that for patients with prostate cancer, > 95% of the PSA isoforms in their serum was found to be in the form of the PSA-ACT complex. At tPSA levels of > 1,000 ng/mL, any free PSA is practically undetectable. However, there is a much greater percentage of free PSA in the serum from patients with benign prostate hyperplasia (BPH). We even detected > 90% free PSA in some specimens. Babaian

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et al. (8) first reported the correlation among serum PSA, prostate gland volume, and age. The individual serum PSA value as a function of the prostate volume was also confirmed by Oesterling et al. (9) and by Hammerer et al. (10), who realized that the enlargement of the prostate gland accounted for the increased serum PSA values in normal older men. All these findings suggested to us that it was the PSA-ACT, not the tPSA that was more tumor specific and that the former's serum concentration would reflect more closely the activity of prostate tumor.

There have been numerous reports appearing in recent literature suggesting that the determination of fPSA and the expression of fPSA as % fPSA helps to improve the specificity of serum PSA tests during cancer screening (5,10–12). In particular, many of the BPH patients identifiable by their high % fPSA could avoid unnecessary biopsies. The measurement of the other serum isoform, the PSA-ACT complex, should aid efforts at cancer detection for similar reasons. There would be less overlap perceived between BPH and prostate cancer if only the PSA-ACT complex were measured. Excluding fPSA from the tPSA concentration diminishes any BPH specimen-associated "PSA values" and creates a greater separation from the values of prostate cancer patients. The amount of fPSA included in the tPSA determination may contribute to distorted prognoses. As pointed out by Catalona et al. (13), the measurement of the % fPSA gave predictive information about the presence or absence of prostate cancer above that provided by other clinical indexes such as age, tPSA level, suspicious results by rectal examination, and prostate size. In fact, their results indicated that there is a systematically decreasing chance of developing cancer with the observance of an increasing % fPSA. Therefore, it should be pointed out that the increase of fPSA and increase of PSA-ACT concentration in the serum carry different clinical meanings. The former is associated with a good prognosis (enlargement of the transition zone of the gland) and latter poor prognosis (tumor progression). A specific measurement of the PSA-ACT complex would avoid common misinterpretations of tPSA results.

The proposal of replacing serum tPSA with specific PSA-ACT assay is also supported by our earlier study, which indicated that overall there is an excellent correlation between serum concentrations of tPSA and PSA-ACT complex in either random and serial specimens from patients with prostate cancer (14). The importance of measuring serum PSA-ACT complex instead of serum tPSA has been recognized by various investigators (15–18). However, technical problems associated with this assay, such as high assay background, discouraged us from recommending the assay as a routine test (19). Recently we found a way to eliminate the high assay background for the PSA-ACT assay. This should eventually allow us to use PSA-ACT assay routinely (20). The problems of antibody selection and calibrator preparation associated with the tPSA are greatly simplified with the establishment of the PSA-ACT assay. With these problems neu-

tralized, it becomes possible for the diagnostic industry to come up with assays that will produce close to identical PSA-ACT values. It has been known for several years that most commercial serum PSA assay kits do not produce identical values for the same specimens (1,21). Using a ratio of fPSA to PSA-ACT instead of fPSA to tPSA also eliminates variations in % fPSA calculation due to any variation of tPSA values from different commercial tPSA assays. Replacing tPSA with the PSA-ACT assay also appears to provide a better differentiation between BPH from prostate cancer during screening. We also found that if patients with prostate cancer or BPH were monitored with specific fPSA and PSA-ACT assays, it will avoid misinterpretation if tPSA assay was used.

MATERIALS AND METHODS

The serum tPSA was measured with three different commercial PSA kits including Hybritech Tandem-E immunoassay kit (San Diego, CA), DPC PSA kit (Diagnostic Product Corp., Los Angeles, CA), and Abbott PSA kit (Chicago, IL) on IMx instrument.

In-House PSA-ACT and fPSA Assay

Both fPSA and PSA-ACT assays were developed in house on the microplate following the sandwich format. Polyclonal anti-PSA antibodies were used in both assays to coat the microwell and to trap the analytes. The trapped fPSA was detected by monoclonal anti-fPSA antibody and the captured PSA-ACT complex was quantified by monoclonal anti-ACT antibody. Our fPSA assay can detect as low as 0.06 ng/mL of fPSA and the cross-reactivity with PSA-ACT is only 0.27%, which enables us accurately to measure the serum fPSA in the presence of much higher serum concentrations of PSA-ACT complex (20,22). Our PSA-ACT assay also has very low cross-reactivity with ACT that the excess amount of ACT present in the serum does not affect the measurement of serum PSA-ACT complex.

Clinical Specimens

All specimens were obtained from the Department of Urology, School of Medicine, University of Utah Health Science Complex. Diagnoses of these specimens had been verified by clinical biopsies. The total PSA was determined on fresh specimens on IMx instrument using the Abbot PSA kit. Frozen specimens were used for the measurement of fPSA, PSA-ACT, DPC tPSA, and Hybritech tPSA at the same time a few months or few years later depending on when specimens were collected, which was especially true for serial specimens collected. The majority of the specimens were obtained from patients with diagnosis confirmed by biopsy.

RESULTS

Elimination of Contributions From Unknown PSA Isoforms

We found that the serum concentration of tPSA of the majority of the random specimens from either patients with prostate cancer or BPH was greater than the sum of serum PSA plus PSA-ACT complex concentrations. The tPSA values shown in Figure 1 were assayed with the Hybritech Tandem E kits. What are shown in Figure 1 are specimens containing Hybritech tPSA within the 3–15 ng/mL concentration range. Apparently many other PSA isoforms present in the serum, in addition to the fPSA and PSA-ACT complex, contributed to the values determined by the tPSA kit. The difference between the concentrations of tPSA and the sum was not a constant. Therefore it will be difficult to apply a correction factor to convert one value to the other. The major problem associated with this difference was our lack of understanding of the clinical meaning associated with changes of the concentrations of these minor serum PSA isoforms. The specific measurements of PSA-ACT specifically can avoid these problems.

Need to Establish Different %fPSA Cutoffs When Using Different tPSA Kits

Calculation of %fPSA is commonly made by dividing the concentration of fPSA by the concentration of tPSA and multiplying by 100. In other words, the calculated % fPSA value is affected by both concentrations of fPSA and tPSA. We selected tPSA kits from a few major diagnostic companies to find out whether same tPSA values are now produced by these commercial kits. As shown in Figure 2, the correlation between Abbott tPSA and Hybritech tPSA concentrations and the correlation between Hybritech and DPC tPSA values were all very good, with correlation coefficients of 0.9 and 0.935 in the tPSA concentration range 0–20 ng/mL, respectively.

Fig. 1. Concentrations of serum tPSA are usually greater than the sum of the serum concentrations of fPSA plus PSA-ACT complex of the same specimens. Comparisons were made by plotting the sum of fPSA and PSA-ACT concentrations against a gradual increase of concentrations of tPSA.

Fig. 2. Correlation of the tPSA concentrations between Abbott and Hybritech assay, and between Hybritech and DPC assay by linear regression analysis.

However, the Abbott tPSA values were generally higher as reflected in the slope (0.824) for the linear regression plot for the correlation between Hybritech and Abbott tPSA concentrations. Less difference was found between the DPC and Hybritech tPSA values. The slope was closer to 1 (0.937). What was less desirable was the fact that there was considerable scattering in both plots. Consequently, any clinical decision made on the basis of these tPSA values could be misleading. For example, a tPSA values of 3 (within upper normal limit) determined by the Hybritech assay could be 5 or higher (elevated) by Abbott kit (due to biased correlation), or 5 or higher by DPC kit (based on the scattering observed).

When portions of these same data, between 0–10 ng/mL, were replotted in Figure 3, based on the gradual increased Hybritech tPSA concentrations, it was clearly shown that Abbott values were ~ one-third higher. However, the scatter-

Fig. 3. Total PSA values determined by the Abbott assay are generally higher than values derived from the Hybritech kit using serum specimens from either BPH patients or patients with prostate cancer (CAP). Only specimens containing Hybritech tPSA concentrations in the range of 4–10 ng/mL were used in this study.

ing shown in Figure 3 does not allow the application of a correction factor to convert fPSA values determined by one kit to values corresponding to the other assay. When we calculated the % fPSA using these Hybritech and Abbott tPSA values (as shown in Fig. 4, Tables 1 and 2) the effect of tPSA concentrations on the % fPSA by two different commercial kits can be readily appreciated. Particularly, a value of 20% fPSA (based on Hybritech tPSA), frequently used as a cutoff to differentiate BPH from prostate cancer, would have been as low as 10% if the Abbott assay was used for calculation. There were less differences between % fPSA calculated with Hybritech and DPC derived tPSA values (Fig. 5). However, some of the scattering of % fPSA values shown in Figure 5 need to be minimized to avoid misinterpretation of the results. Conceivably, same PSA-ACT values can be produced by various commercial kits to avoid these problems.

Avoiding Misinterpretations Based on tPSA Values

During treatment, patients are generally monitored by the measurement of certain tumor marker. The increase and decrease of serum tumor marker concentrations serve as an indication of the progression and remission of the tumor or the success and failure of the treatment. However, when we measured the specific fPSA and PSA-ACT complex simultaneously, it was evident to us that any clini-

Fig. 4. % fPSAs calculated from Abbott tPSA were generally lower than the % fPSA calculated from Hybritech values.

cal interpretation based on the changes of tPSA values could be misleading. Changes in serum fPSA or PSA-ACT concentration are known now to relate specifically to the change of the prostate volume or tumor activity, respectively. Based on the changes of fPSA and PSA-ACT serum concentrations of the same serial specimens monitored on tPSA, we could tell that interpretations based on changes of the serum tPSA can be misleading. Discussed below are examples of what can go wrong if the interpretation is based on the serum concentration of tPSA.

1. Changes in tPSA differ from change in PSA-ACT concentrations

As shown in Figure 6, changes of the serum concentrations of tPSA in these three patients did not correspond properly with the changes occurred in PSA-ACT complex and fPSA. Because the tPSA concentrations were determined with the Abbott kit, we expected them to be slightly higher than the sum of concentrations of fPSA and PSA-

TABLE 1. Based on Hybritech Tandem-E tPSA Assay

	Cutoff = 16.5%	Cutoff = 6%
CAP patients (N = 233)	90% < 16.5%	55% < 6%
BPH patients (N = 151)	30% < 16.5%	90% > 6%
Consequence	30% BPH patients may spare biopsy	55% cancer patients can be identified

TABLE 2. Based on Abbott tPSA Assay

	Cutoff = 13.5%	Cutoff = 5.8%
CAP patients (N = 233)	90% < 13.5%	60% < 5.8%
BPH patients (N = 151)	22% < 13.5%	90% > 5.8%
Consequence	22% BPH patients may spare biopsy	55% cancer patients can be identified

ACT complex, both standardized against Hybritech assay. However, in patients S19 and S197, the combined concentration of fPSA and PSA-ACT was far too low than the tPSA concentration. Apparently other unknown PSA isoforms also contributed to the tPSA concentration shown in Figure 6. Most important is the fact that in patient S197, for example, the slight increase of tPSA was due to the increase of fPSA and not the PSA-ACT complex. If the specific PSA-ACT concentration were not monitored, the discrepancy would not have been detected and the results would have been misinterpreted.

Fig. 5. Comparison between the % fPSAs calculated from the Hybritech and that from the DPC tPSA assay.

Fig. 6. Changes of the tPSA concentration disagreed with the changes of the serum level of PSA-ACT. Patients with prostate cancer were monitored with fPSA, tPSA and PSA-ACT assays during treatment.

2. Changes of tPSA correlate with changes in both fPSA and PSA-ACT concentration

In Figure 7, we demonstrated that occasionally we found that both changes of fPSA and PSA-ACT parallel changes observed in serum tPSA. Whereas the increase of tPSA and PSA-ACT are thought to be associated with poor prognosis or tumor progression, the increase of fPSA is associated with prostate volume change. An inspection of tPSA concentrations alone may lead to speculations about the presence of a fast progressing tumor due to the rapid increase of tPSA. One would remain unaware of the fact that the rapid increase was contributed by an increase in fPSA. Monitoring tPSA alone would not provide us with this crucial and specific information.

3. Changes of tPSA were due to changes occurred in PSA-ACT concentrations

There were cases in which changes observed in tPSA was largely derived from changes of the PSA-ACT concentration (Fig. 8). These were cases in which the specific measurement of the PSA-ACT did not provide any additional information.

Better Differentiation of BPH from Prostate Cancer

1. Measurement of PSA-ACT at tPSA between 4–10 ng/mL

It is well known that most BPH specimens overlap with prostate cancer in the tPSA concentration range of 4–10

Fig. 7. Changes of serum tPSA concentration parallel to both changes of serum level of PSA-ACT and fPSA.

ng/mL. It also has been found recently that BPH patients contain more fPSA or less PSA-ACT concentrations in their sera compared to serum samples from prostate cancer patients. Conceivably, the specific measurement of the PSA-ACT complex will provide a better differentiation between BPH and prostate cancer. We selected 167 and 123 specimens from patients with prostate cancer and BPH, respectively, with tPSA in the range of 4–15 ng/mL and measured their PSA-ACT complex concentrations. The results are shown in Table 3. As indicated, the difference in the average values between cancer patients and patients with BPH is much larger in PSA-ACT (6.1 ± 2.9 vs. 4.2 ± 2.4 ng/mL) than in tPSA (8.0 ± 2.8 vs. 7.1 ± 2.5 ng/mL).

2. Replacing tPSA with PSA-ACT for % fPSA calculation

Percent fPSA has been calculated as the ratio of fPSA/tPSA then multiplied by 100. We found if the tPSA concentration was replaced by the concentration of PSA-ACT, the resulting % fPSA provided better differentiation of BPH from prostate cancer. Because of the smaller values of PSA-ACT associated with BPH the % fPSA calculated for BPH using PSA-ACT instead of tPSA would make % fPSA of BPH much

Fig. 8. Changes of serum tPSA concentration parallel to only changes of serum level of PSA-ACT.

larger. However, because in prostate cancer patients the concentrations of PSA-ACT are not much different than the tPSA concentrations, less change will be noted for % fPSA of cancer specimen resulted in larger difference between BPH and prostate cancer. The results are shown in Figure 9.

Although in general BPH specimens contain more fPSA, it is the percent fPSA that is higher in BPH not the absolute amount of fPSA. Therefore, fPSA concentration in BPH can be less than what are found in cancer specimens because in general the tPSA in cancer specimens is much higher than the tPSA found with BPH patients. In other words, serum concentration of fPSA alone does not differentiate BPH from prostate cancer. Therefore, we need to express fPSA in terms of tPSA or as ratio of fPSA to PSA-ACT in order effectively to differentiate BPH from prostate cancer.

TABLE 3. Concentration of PSA-ACT Complex of BPH and CAP Patients Containing Various Ranges of Concentration of tPSA

Diagnosis	tPSA Conc. Range (ng/mL) ^a	Average tPSA (ng/mL)	Average Conc. Of PSA-ACT (ng/mL)	# of specimens
CAP	4–280	2.1 ± 21.4	9 ± 12.7	194
CAP	4–15	8.0 ± 2.8	6.1 ± 2.9	167
CAP	4–20	8.6 ± 3.6	6.7 ± 3.6	151
BPH	4–15	7.1 ± 2.5	4.2 ± 2.4	123
Normal	0–3.4	1.9 ± 1.3	1.7 ± 1.3	45

^atPSA concentration was based on Abbott assay.

Free PSA/tPSA × 100 or % fPSA has been used to express fPSA and used to differentiate BPH from prostate cancer to eliminate many unnecessary biopsies. However, as discussed above, because different commercial kits may still produce different tPSA values for the same specimens, the % fPSA calculated will vary according to the tPSA kit was used to obtain the tPSA value. This problem can be eliminated if we express fPSA in terms of fPSA/PSA-ACT × 100. It is much easier for all commercial kits to produce identical values for PSA-ACT. Expressing fPSA as fPSA/PSA-ACT × 100 also allow better differentiation of BPH from prostate cancer because of the presence of more fPSA in BPH.

DISCUSSION

The problem associated with the tPSA assay has been recognized for years. Despite efforts to improve the calibrator preparation and antibody selection by diagnostic industries, the values of tPSA produced by many different commercial kits are still not all the same on the same specimens.

The proposal of “equal molar reactivity” provided a good guidance for assay improvement, but failed to provide a complete solution to the problem. We tested only a limited number of commercial tPSA kits that were available to us at the

time. We were surprised to find that the discrepancy still exists with major diagnostic companies. When clinicians are relating precise values of tPSA in various clinical situations, especially during cancer screening and using % fPSA to differentiate BPH from prostate cancer, it should be a major concern that, in reality, all these values were not that reliable. The failure to reach a satisfactory agreement among various commercial kits for years suggests that the problems associated with the tPSA assay will not be easily removed. It is perhaps time we sought out alternatives.

The importance of developing an assay specific for serum PSA-ACT complex has been pointed out by various investigators. One of the reasons that the assay has not become popular is because there was a major technical problem associated with the assay. A very high assay background was frequently experienced by investigators using a sandwich, two antibodies immunoassay format. Apparently, it was found that anti-ACT detecting antibody used for the assay also reacted with ACT-cathepsin G complex bound to the solid phase (or microwell) and gave a very high assay background that produced falsely positive values. However, we recently found a way to eliminate this high assay background, and it appears that the assay is ready for routine clinical use.

Fig. 9. Expressing fPSA as “fPSA/PSA-ACT × 100” provides better differentiation between BPH and prostate cancer.

With both fPSA and PSA-ACT assays available, it became possible for us to identify many advantages when the tPSA assay was replaced by the PSA-ACT assay. Although only two major PSA isoforms, namely, the fPSA and PSA-ACT complex, are immunologically detectable, many other serum PSA isoforms also contribute to the tPSA value produced by the tPSA assay. The detecting anti-PSA antibody can react with all PSA isoforms in varying degrees; therefore, all PSA isoforms contribute to the final PSA-ACT value. Even with the PSA-A2M (PSA- α_2 -macroglobulin) complex, we actually found, contrary to what is commonly believed, low immunological reactivity with PSA-A2M complex with the tPSA assay. The problem with these unknown serum PSA isoforms is related to the fact that we do not know how their serum concentrations relate to the tumor activity and clinical status of the patients. We also have no way of knowing how much these individual isoforms contribute to the tPSA value. Therefore, the best approach is not to include them in the assay and, for example, specifically to measure the PSA-ACT complex.

It is possible to differentiate BPH from prostate cancer by the determination of % fPSA because BPH serum specimens contain much more fPSA than cancer samples do. In other words, a much higher percentage of PSA-ACT complex is associated with cancer specimens than with specimens from BPH. Therefore, replacing the tPSA assay with the PSA-ACT assay should improve our ability to differentiate BPH from prostate cancer during screening for prostate cancer at the tPSA concentration range of 4–10 ng/mL. In addition, because the difference between the average PSA-ACT concentration of BPH patients and that of cancer patients was greater than the difference in tPSA between these two groups of specimens, replacing tPSA with PSA-ACT for the calculation of % fPSA also appeared to enlarge the difference between BPH and prostate cancer. This can be easily explained. When the same fPSA value from the cancer patient is divided by the tPSA or PSA-ACT, there will be little difference in % fPSA values derived from both calculations in cancer specimens due to the small difference of tPSA and PSA-ACT concentration in those samples. However, larger differences between tPSA and PSA-ACT concentrations exist in BPH specimens because of the presence of a higher percentage of fPSA with the BPH samples. As a result, the % fPSA expressed based on the PSA-ACT concentration enlarges the difference between BPH and prostate cancer, as shown in Figure 9.

Use of % fPSA has become increasingly popular since Catalona et al. (13) expressed fPSA as % fPSA to help differentiate BPH from prostate cancer and to avoid many unnecessary biopsies. Since the calculation of % fPSA involves the tPSA, the precision of tPSA determination can have a considerable impact on the % fPSA calculated. Even at the present time, many clinicians still may not be aware of the fact that the tPSA values produced by different commercial kits may not agree with each other (1996 proficiency test by New York State clearly demonstrated the discrepancies still exist among

all the major kits despite efforts to develop equi-molar assays by various companies). Since the % fPSA derived from different tPSA kits will be different, clinicians should know which tPSA kit the laboratory is using to produce the tPSA value and ensure that the kit used for their patients is the same kit used in the literature where they adopted the cut-off values for % PSA. In contrast, if the tPSA value for the % fPSA calculation is replaced by the value of PSA-ACT assay, such a problem may be effectively eliminated. In addition, the % fPSA calculated from the PSA-ACT concentration expands the difference between BPH and prostate cancer.

PSA density has been one of the approaches to improve the test specificity of tPSA assay (23). By using the ratio of the serum tPSA concentration to prostate volume, it provides better distinction between BPH and prostate cancer. Recently, it was found that the discrimination between BPH and prostate cancer could be further improved by using PSA density specifically of the transition zone (24). The average PSA density of the transition zone was determined to be 0.21 ± 0.13 and 1.02 ± 0.7 ng/mL/cc, respectively, for patients with BPH and prostate cancer. PSA density of the transition zone, therefore, could become a routine tool for urologists in the prediction of prostate cancer in men with a PSA of 4–10 ng/mL. We feel that the difference between BPH and prostate cancer can be further expanded if the tPSA is replaced by PSA-ACT concentration. The lower and higher percent of serum PSA-ACT concentration in BPH and prostate cancer, respectively, would make the value of PSA density smaller for BPH and larger for prostate cancer.

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