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PROSTATE SPECIFIC ANTIGEN

A review

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Abstract

Despite its identification in the 1970s, prostate specific antigen (PSA) has only recently gained widespread utility. In this review, we will describe significant aspects of the biochemistry of PSA, immunohistochemical applications, the effect of prostatic manipulation on the serum level of this analyte, and other considerations in the determination of the level of PSA. In addition, the effect of benign prostatic hyperplasia (BPH) on serum PSA will be described. Finally, the application of PSA in the detection, staging and monitoring of patients with prostatic carcinoma will be discussed.

Key words: Prostate, cancer, prostate specific antigen, review.

Prostate specific antigen (PSA) is a major advance in our urologic tumor marker armamentarium. For a variety of reasons, this analyte represents a significantly better serum marker than prostatic acid phosphatase (PAP), and many regard PSA to be the best existing marker for prostate malignancy. In this review, several salient features of PSA will be highlighted, with special emphasis on the biochemistry of this protein, its immunohistochemical implications, serum assays, clinical considerations in the measurement of PSA, the relationship of PSA to benign prostatic hyperplasia (BPH), and its usefulness in the management of patients with prostatic carcinoma.

History

Although most reports have credited Wang et al. (1) with the development of PSA, it is important to recognize the early work of Li & Beling (2) and Sensabaugh (3) for early characterization of the protein which subsequently became known as PSA. The numerous publications of Wang et al. at Roswell Park brought it into the clinical realm. Additionally, early reports by groups at the University of Minnesota (4), Johns Hopkins (5) and Stanford University (6) have resulted in widespread clinical application of this marker.

Biochemistry

PSA is a 34 kilodalton glycoprotein which is found only in the cytoplasm of epithelial cells of prostatic origin. Present in both benign and transformed luminal epithelial cells, PSA is detectable in the serum of men who harbor prostatic tissue. The structure and function of PSA is known. PSA is a neutral serum protease and functions to lyse the seminal coagulum following ejaculation (7, 8). PSA is a 240 amino acid protein. It is important to note that PSA is both functionally and immunologically distinct from PAP.

Immunohistochemistry

The tissue specificity of PSA noted above makes it an extremely useful marker for immunohistochemical analysis of tumors of unknown etiology. Numerous authors have investigated primary and metastatic prostatic carcinoma utilizing immunohistochemical preparations with PSA antibodies (9–14). As can be seen in Table 1, virtually all primary and metastatic prostatic carcinomas that have been examined, stained positive with PSA antibodies. Moreover, prostatic carcinoma that has been radiated continues to preserve PSA labeling (13). On the other

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Table 1

PSA immunohistochemistry

Author	Primary CAP[1]	Metastatic CAP	Irradiated CAP	Other CA[2]
Nadji et al. (9)	73/73	49/49		0/78
Stein et al. (10)	13/15			
Vernon et al. (11)	30/30		5/5	
Ford et al. (12)	63/65	16/17		0/13
Brawer et al. (13)			33/33	
Sohlberg et al. (14)	23/23			
	202/206	65/66	38/38	0/91

[1]CAP prostate carcinoma
[2]Non-prostate carcinoma

hand, non-prostatic malignancies have been shown to be non-immunoreactive with antibodies to PSA as have studies of benign nonprostatic tissue.

Information derived from PSA immunohistochemistry of tumors of unknown etiology may prove clinically useful. For example, we have recently reported a case in which PSA immunohistochemistry proved prostate as the site of origin for an undifferentiated adenocarcinoma obtained on bronchoscopic biopsy (15). Androgen deprivation therapy resulted in a marked clinical response. We believe that all adenocarcinomas of unknown etiology in men should be evaluated with PSA immunohistochemistry. It should be noted that, frequently, tumor cell heterogeneity is present within a prostatic neoplasm with respect to PSA immunohistochemistry—some cells being minimally immunoreactive. However, we have not observed any case of prostate carcinoma in which no PSA labeling was present (unpublished observation).

Serum assays

The isolation of the PSA protein resulted in the development of numerous antibodies against this molecule. Subsequently, serum assays for the measurement of PSA were developed. Currently, two assays are commercially available: the Tandem-R (Hybritech, Inc., San Diego, CA) and the Pros-Check (Yang Laboratories, Bellevue, WA). Although significantly different, these assays both measure the same analyte and provide useful clinical information. Several analytic differences between these tests are depicted in Table 2.

The Tandem-R assay is a two-site immunoradiometric assay employing two monoclonal antibodies. In contrast, the Pros-Check assay is a conventional radioimmunoassay employing polyclonal serum. Chan et al. (16) and Hortin et al. (17), in an assessment of patient serum assayed with both tests noted an r-value of between 0.95 and 0.99. PSA concentration utilizing the Pros-Check assay generally is between 1.4 and 1.8 times higher in value compared to those measured with the Tandem-R assay. Obviously, this mandates the clinical laboratories' reporting which assay is employed when they give clinicians results of PSA level. It is unclear which assay is more representative of the actual analyte concentration as there is no international standard for PSA. The establishment of such a standard would allow for resolution of these differences in the assay. Nevertheless, it should be emphasized that both assays can be used to provide significant, reproducible data.

Table 2

PSA assay comparison. Refs (4–6, 16–18, 20)

	Tandem-R	Pros-Check
Assay type	Immunometric	Radioimmunoassay
Antibody	Monoclonal	Polyclonal
Normal range (ng/ml)	0.0–4.0	0.0–2.5
Analytical sensitivity (ng/ml)	0.1–0.2	0.1–0.2
Low detectability (ng/ml)	0.1–0.6	0.2–0.3
Linearity (r)	0.95–0.99	
Bias	P-C × 0.6–0.7	T-R × 1.4–1.8
Half life (days)	3.2	2.2

The analytic sensitivity of PSA has been reported to be between 0.1 and 0.2 ng/ml (16, 17). This level is extremely important, since following radical prostatectomy, if complete removal of all prostatic tissue has been achieved, the PSA should fall to non-detectable level (female serum level). This lowest level of detectability has been reported to be between 0.1 and 0.6 ng/ml using the Tandem-R assay (4, 16), and between 0.2 and 0.3 ng/ml for the Pros-Check assay (7, 18). Each facility must establish their own low level of detectability value.

Not infrequently, after radical prostatectomy, the PSA will 'hover' around the low level of detectability. The development of a more sensitive PSA assay would help resolve this dilemma. If a more sensitive assay could be

developed, one could perhaps ascertain at an earlier state, persistance of disease and thus offer earlier adjuvant therapy. Numerous laboratories are currently trying to develop such an improved test.

Normal range

The establishment of normal range for tumor markers generally is achieved by measuring the analyte in a population of normal volunteers and deriving confidence intervals for this population. For most malignancies, it is appropriate to assume, that a healthy population is free of disease and thus constitutes a normal population. Prostatic disease, however, both benign and malignant, is ubiquitous in the older male and is often present in the absence of clinical manifestations. Indeed, autopsy studies have shown that carcinoma is present in more than 30% of 'normal' men and benign prostatic hyperplasia which, as will be discussed, has been implicated in causing elevation of the PSA, is found in most men over age 50. The absence of prostatic carcinoma or benign hyperplasia can only be proven by careful sectioning of the prostate. Obviously, this is impossible in the establishment of a normal population.

Despite these significant problems, the normal range for PSA has been reported by several investigators. Stamey et al. (6), in a study of 157 men utilized the Pros-Check assay and defined the reference range to be less than 2.5 ng/ml. Also utilizing the Pros-Check assay, Chan et al. (16) reported a reference range less than 4.2 ng/ml. With the Tandem-R assay, Myrtle et al. (19) found the reference range to be less than 4 ng/ml and Chan et al. (16) stated that it is less than 2.8 ng/ml. Ercole et al. (4) stated that whereas all men under age 40 had a PSA less than 4 ng/ml, 3% of 'normal' males over age 40 had PSA greater than 4 ng/ml.

Considerations in the measurement of serum PSA level

One of the advantages of PSA compared to PAP is the greater stability of the former. For instance, we evaluated the serum of 9 men which was stored for 24 h at room temperature and noted that, whereas PSA fell only 3.1% from the prestorage level, PAP fell 15.2% (20). In addition to this stability, PSA has less diurnal variation than PAP. One of the problems that has been reported with PAP is the marked fluctuation of the serum level throughout the day (21). In order to evaluate this phenomenon, we performed morning and afternoon PSA determinations on 10 men on three consecutive days and compared PSA and PAP levels utilizing the Tandem-R assay for PSA as well as on PAP (20). Substantially increased variation in PAP levels was noted. Maatman (22) has recently reported similar minimal diurnal variations with PSA. Furthermore, Stamey et al. (6), reported minimal fluctuations when serum PSA was drawn over a 6-week interval. The Stanford group has reported a substantial decline in the PSA after patients are admitted to hospital and these authors stress the importance of making clinical decisions on the basis of ambulatory PSA determinations.

It has been widely reported that digital rectal examination caused spurious elevation of the PAP (23–25). We performed serum PSA and PAP determinations prior to, and 5 and 30 min following standard digital rectal examination in the 26 men being evaluated for a variety of urologic complaints (26). We noted no significant effect of rectal examination either on PSA or PAP level utilizing the Tandem-R assay for both analytes. In contrast, Stamey et al. (6) noted elevation of serum PSA following prostatic massage. These authors utilized the Yang assay, but it is more likely that the difference reflects the greater prostate trauma caused by massage. Stamey et al. noted significant elevations of serum PSA following prostate needle biopsy and transurethral resection-procedures that are associated with significant prostatic trauma (27).

PSA and benign prostatic hyperplasia

A number of authors have reported a significant elevation of the serum PSA level in men with benign prostatic hyperplasia (BPH) (4, 6, 28, 29) (Table 3). Stamey et al. calculated that for every gram of BPH, the serum PSA was elevated 0.3 ng/ml basing this on a simple prostatectomy series (6). In contrast, these authors noted that in prostatic carcinoma, the relationship was 3.5 ng/ml for each gram of carcinoma.

On problem in evaluating the reports of BPH and PSA is the possibility that other significant, but unrecognized, prostatic pathology in addition to the hyperplasia may have contributed to the elevation of the serum PSA level. In the histologic examination of the prostatectomy specimen, the pathologist may have concentrated on excluding incidental carcinoma, and not on other lesions. Other prostatic conditions, including inflammation and prostatic intraepithelial neoplasia (PIN), a putative premalignant change, may not have been reported. Of perhaps greater

Table 3

Serum PSA in patients with histologically confirmed benign prostatic hyperplasia (%)

Author (ref.)	Assay	PSA > 2.5	PSA > 4.0	PSA > 10.0
Ercole et al. (4)	Tandem-R		75 (21)	10 (3)
Ferro et al. (28)	Tandem-R			13 (33)
Hudson et al. (29)	Tandem-R		35 (21)	3 (2)
Stamey et al. (6)	Pros-Check	70 (88)		

significance is the recognition that BPH develops in the transition zone described by McNeal (30). The vast majority of prostatic carcinomas arise in the peripheral zone, which is only minimally, if at all, sampled in simple prostatectomy specimens.

There are two requirements for PSA to be detected in serum. Obviously, PSA secreting cells must be present in the patient. The increased serum PSA in patients with carcinoma may simply reflect a relatively increased number of cells with PSA. Of equal importance, however, is that this analyte of not inconsequential size, must gain access to the systemic circulation before it can be detected. The anatomy of the prostate is such that several potentially formidable barriers are situated between the prostate luminal cells, where PSA is elaborated, and the surrounding capillaries. Interposed between these structures are the prostate basal cell layer and basement membrane, the intervening stroma, the capillary basement membrane, and endothelial cells. BPH, while elaborating considerable amounts of PSA, has an intact basal cell layer as well as basement membrane. One explanation of why PAP levels are significantly higher than PSA, is that the former is a much smaller molecule and therefore may more readily gain access to the systemic circulation. It was the recognition of these layers in hyperplastic tissue that prompted us to investigate the underlying histology in men undergoing simple prostatectomy vis-à-vis their serum PSA level.

In this study, we performed detailed histologic examination of all tissue removed from 81 men undergoing simple prostatectomy for presumed benign bladder outlet obstruction (31). Specifically, we searched for incidental carcinoma, PIN, and acute and chronic inflammation. PIN is a lesion comprised of cytologic atypia and proliferation of the normal single luminal layer of the prostatic acini and ductules. PIN fulfills the majority of requirements for premalignant change (32, 33). Our previous observations of disruption of the basal cell layer in PIN was the motivation for searching for this lesion in the simple prostatectomy specimens (32). PSA was evaluated utilizing the Tandem-R assay.

In this investigation, 36 men had PSA above 4.0 ng/ml, the established upper limits for this assay. Among these, there were 11 carconomas, 13 patients with PIN and 11 with acute inflammation. Of the 26 patients with simple hyperplasia alone noted in their specimen, only one had a PSA greater than 40 ng/ml. We concluded that serum PSA was rarely elevated in patients with simple hyperplasia alone, in contrast with the reports from other investigators.

In an effort to obtain more data in this regard, we recently compared the serum PSA level in men undergoing ultrasound-guided prostate needle biopsy of palpable prostatic abnormality with the histology obtained. Obviously, needle biopsy results in more limited sampling than that found in the simple prostatectomy specimens. One advantage of needle biopsies, however, is the fact that the majority of prostatic diseases including carcinoma, PIN, and inflammation occur in the peripheral zone which is more selectively sampled in this approach.

Among 155 men undergoing this procedure, 78 (50%) had benign histology. Their mean PSA was 3.6. No difference in PSA was discernable between those with chronic inflammation and those with other benign histology. Among 22 patients (14%) with PIN, the mean PSA was 5.9. The five patients with PIN grade 1 (our lowest grade), had a mean PSA of 1.9. Of the 17 patients with higher grade PIN (grade 2 or 3), the mean PSA was 7.0. Fifty-five patients (36%) in this series had carcinoma. These individuals had a mean PSA of 17.9. Again, these data suggest that the correlation between prostatic histology and elevation in serum PSA may be associated with disruption of the basal cell layer (PIN and carcinoma). Alternatively, and perhaps of greater likelihood is that the PSA elevation may be a reflection of the common association of PIN and carcinoma.

This latter hypothesis is substantiated by our recent evaluation of men undergoing ultrasound-guided prostate needle biopsy who had PIN noted on a previous biopsy (34). In a compilation of 18 such patients, carcinoma was noted on the subsequent biopsy in 12 (67%). Twelve patients had PSA values obtained prior to the second biopsy. Nine of these had carcinoma on the second procedure, and their mean PSA was 11.7 (range 0.5–33.6). Of the three patients with non-carcinoma on the repeat biopsy, the mean PSA was 2.0, range 1.6–2.6.

In an effort to further evaluate the relationship of PIN and carcinoma, we have performed a quantitative immunohistochemical study on 23 men undergoing radical prostatectomy (14). In this evaluation, PSA antibodies were used to stain sections from the radical prostatectomy specimens in which benign tissue and PIN as well as carcinoma was present on the same histologic preparation. We noted significantly decreased immunoreactivity with PSA in both carcinoma and PIN compared with the benign tissue which served as an internal control. This study suggests that it is not an increased production of PSA in both PIN and carcinoma that is associated with increased serum levels, but rather an increased disruption of the tissue layers described above.

PSA and prostate cancer diagnosis

The ability of serum PSA to detect prostatic carcinoma is undergoing extensive investigation. Currently, no data are available in which serum PSA level alone was used to identify patients for further workup for the detection of carcinoma.

We can obtain some information by looking at PSA performance in patients with an established diagnosis of carcinoma. Myrtle et al. (19) evaluated patients with

clinically staged prostatic carcinoma and noted abnormal serum PSA levels (Tandem-R assay) in 63% of patients with stage A carcinoma, 71% of those with stage B, 81% of stage C patients and 88% of patients with stage D disease. In contrast, the serum PAP was abnormal in only 12% of patients with stage A disease, 22% of those with stage B, 38% with stage C and 67% of those with stage D carcinoma. Stamey (27), utilizing the Yang assay, reported that 48% of patients with stage A1 disease had abnormal PSA, 57% with stage A2, 95% of stage B and 100% of stages C and D had abnormal PSA. In contrast, only 14% of stage A, 32% of stage B, 64% of stage C and 89% of stage D had abnormal PAP. The apparent improved sensitivity of PSA for the detection of carcinoma in the series of Stamey as compared to that of Myrtle et al. may reflect differing cut-off values of normality and not intrinsive differences in the assay utilized.

Table 4 illustrates data from several series describing preoperative serum PSA concentration in patients undergoing radical prostatectomy. As can be seen, utilizing the Tandem-R assay with the upper range of normal as 4.0 ng/ml, between 43 and 85% of patients undergoing radical prostatectomy have an abnormal PSA (29, 35–37). With the Pros-Check assay, Stamey et al. (38) have reported abnormal PSA in 91% of patients.

While these series offer some insight into the performance characteristics of PSA for the detection of carcinoma, it must be emphasized that in all these reports, carcinoma had been established by other means. The use of these data to speculate as to the performance of this assay for detection of carcinoma is controversial. There is a strong correlation between PSA level and carcinoma stage which will be discussed subsequently. Patients who come to clinical diagnosis of carcinoma may indeed have more advanced stage than others with smaller volumes of carcinoma. Thus, utilizing radical prostatectomy or other series in which the diagnosis of carcinoma has been ascertained to speculate as to the ability of PSA to detect carcinoma, may result in abnormally high expectations. On the other hand, the clinically detected population may indeed represent a meaningful group to assess the utility of PSA. These are precisely the patients who we wish to treat since they have neither such insignificant cancer volumes that they most likely need no treatment nor widespread metastatic disease so that they have been excluded by clinical parameters.

Another approach to identify the performance of PSA characteristics is to look at its ability to predict carcinoma in men undergoing simple prostatectomy. In our simple prostatectomy series described above, we noted an abnormal PSA in 71% of those patients who were found to have carcinoma using the Tandem-R assay and a cut-off value of 4.0 ng/ml (31).

Another population that can be used to ascertain PSA performance charateristics for the detection of carcinoma is among those men undergoing ultrasound-guided prostate needle biopsy. Table 5 illustrates Tandem-R PSA performance characteristics utilizing a 4.0 ng/ml cut-off in the series reported by Cooner and among our own patients. It should be emphasized that in Cooner's series, it was the presence of a sonographic abnormality which prompted the biopsy. In our own series, we

Table 4

Preoperative PSA and radical prostatectomy

Author (ref.)	Assay	n	%PSA >		
			2.8 ng/ml	4.0 ng/ml	10.0 ng/ml
Lange et al. (35)	Tandem-R	100	84	71	41
Partin et al. (36)	Tandem-R	350	71	43	
Hudson et al. (29)	Tandem-R	41		85	29
Brawer et al. (37)	Tandem-R	60	80	78	30
Total	Tandem-R	551	75	55	29
Stamey et al. (38)	Pros-Check	102	91		

Table 5

PSA performance in carcinoma detection in patients undergoing ultrasound-guided prostate biopsy

Author (ref.)	n	Carcinoma n (%)	PSA > 4.0 ng/ml		Positive predictive value
			Sensitivity	Specificity	
Cooner (unpubl.)	835	263 (32)	80	61	48
Brawer (unpubl.)	246	79 (32)	65	69	50

performed ultrasound-guided biopsy in all men with an abnormal digital rectal examination, ranging from benign-feeling asymmetry to a frank nodule. The slight differences in sensitivity, specificity and positive predictive value noted among the two series may reflect these differences in what initiated biopsy. As is shown, Cooner noted a sensitivity of 80%, a specificity of 61%, and a positive predictive value of 48%. Similarly, our data reveal a sensitivity of 65%, a specificity of 69%, and a positive predictive value of 50%.

The association of serum PSA and prostatic intraepithelial neoplasia (PIN), has been discussed above. Furthermore, as described, we have noted a correlation between PIN, noted on prostate biopsy, and the subsequent diagnosis of carcinoma on repeat biopsy. These observations suggest that the ability of PSA to be useful in detecting carcinoma may be even greater than that suggested by the data in Table 5.

PSA and the staging of prostatic carcinoma

One of the great problems in the management of patients with apparently localized prostatic carcinoma is the frequent upstaging noted at radical prostatectomy. Despite improved imaging modalities, including prostatic ultrasound and magnetic resonance, the ability to accurately predict pathologic stage is lacking. The correlation between serum PSA level and pathologic stage has been recorded by numerous investigators. Several series are depicted in Table 6. As is readily apparent, there is a strong correlation between the pathologic stage and serum PSA level when a large number of patients are evaluated. However, substantial 'scatter' within any pathologic stage and overlap between stages in the range of PSA precludes the use of the preoperative PSA level alone to make treatment decisions. Perhaps the use of additional markers for malignant behavior, such as tumor volume as assessed by transrectal ultrasound, histologic grade of needle biopsy specimen and DNA ploidy may be used in concert with the serum PSA to more accurately predict the preoperative stage.

PSA in the monitoring of patients with prostatic carcinoma

The greatest utility of PSA today is in the monitoring of patients with prostaic carcinoma. The complete tissue specificity of this analyte makes this application particularly useful in men treated with radical prostatectomy. Following successful removal of all prostatic tissue, the PSA should fall to the undetectable level. Thus, it is important for each clinical laboratory to ascertain this level. With increasingly advanced local stage, a higher percentage of patients have persistent PSA following radical prostatectomy (4–6, 29, 35, 37, 38).

In the series of Lange et al. (35), clinical evidence of progression occurred in only 9% of patients who had PSA less than 0.4 ng/ml three months following surgery, in contrast to progression in all patients whose PSA was greater than 0.4 ng/ml. The usefulness of persistent PSA following radical prostatectomy for adjuvant radiotherapy has been described by Lange et al. (35), and Link et al. (39). Lange et al. describe PSA falling to the undetectable level following adjuvant radiation therapy in 53% of patients (35). In the series by Link et al., the PSA fell in 62% of patients to <0.3 ng/ml, however, they noted that in only two of the patients did the PSA remain at the undetectable level for more than one year (39). Further investigation in the use of PSA to identify patients for adjuvant therapy following radical prostatectomy is clearly warranted.

Little data exists on the effect of primary external beam radiotherapy on serum PSA. Stamey et al. (40), reported that following definitive radiotherapy, the PSA fell to the normal range in 36% of patients. It fell to the undetectable level in 11%. Hudson et al. (29) noted a fall to the undetectable level in 3 of 18 patients. Disappointingly, however, Stamey et al. (40), noted than 12 months following definitive radiotherapy, the PSA was continuing to fall in only 8% of patients, was stable in 41%, but was rising in 51% of 80 patients examined.

Oftentimes, PSA falls dramatically following androgen ablation therapy in patients with stage D carcinoma. Moreover, Ercole et al. (4) and Hudson et al. (29) correlated the magnitude of fall of PSA with response to therapy. Stamey et al. (41) noted that 9% of 45 patients

Table 6

PSA and pathologic stage (%PSA > 10.0 ng/ml)

Author (ref.)	Assay	Organ confined	Capsular penetration	Seminal vesicle invasion	Lymph node metastases
Ercole et al. (4)	Tandem-R	7	32	100	71
Hudson et al. (29)	Tandem-R	11	5	0	100
Oesterling et al. (5)	Tandem-R	10	20	61	71
Stamey et al. (6)	Pros-Check		82	95	100

treated with androgen ablation therapy had a PSA fall down to the undetectable range. An additional 13% fell to below normal. Among 36 patients followed serially with PSA values, the level continued to fall in 19% of patients treated with androgen ablation therapy, but was rising in 67% (14% stable) (41).

Conclusion

PSA represents a major tool in the management of patients with prostatic disease. It has tremendous application in the areas of immunohistochemistry and monitoring of patients with established prostatic carcinoma. The utility of PSA for the early detection and staging of prostate carcinoma remains controversial. Further investigation in this area is necessary and is under way. It is our contention the PSA has largely supplanted PAP in the management of patients with prostatic carcinoma.

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