REVIEW ARTICLE

Prostate Specific Antigen (PSA):The Historical Perspective

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INTRODUCTION

In 1970, shortly after joining Roswell Park Memorial Institute, the New York State institute for the study of malignant diseases, the author initiated investigations on the use of tumor cell products for diagnosis and therapy of cancer. Immunochemical approaches were used primarily to differentiate quantitatively or qualitatively normal cells from tumor cells. Prostate cancer was a major area of endeavor, with the goal to identify and characterize prostate tumor specific and associated antigens, and eventually to develop a simple but reliable blood test for prostate cancer. Prostate cancer research had not received much attention at the time this work was begun. The early studies focused upon, among others, prostatic acid phosphatase, alkaline phosphatase, and new prostate tumor markers. By the mid 1970s, three able investigators--Dr. Ching-Li Lee, Dr. Carl S. Killian, and Dr. Ming C. Wang--had joined the prostate cancer research team, and were invited to take charge of these three research projects, respectively.

DISCOVERY AND PURIFICATION

Years of hard work eventually resulted in the publication of the team's first paper reporting the discovery and purification of PSA in 1979 (1). A key reagent in this process was an antiserum (designated p8) that was generated in 1975 in rabbits using an extract of prostate tumor as the immunogen. Using specimens from normal, benign hyperplastic, or cancerous prostate tissues, and after absorbing the antiserum with normal serum from young female rabbits, two precipitation lines were detected by immunoelectrophoresis (IEP) in each case. One of the two precipitins was later identified as prostatic acid phosphatase (PAP), and the other as PSA (or at the time abbreviated as PA), a new prostate specific antigen. After the antibodies reactive with PAP were removed, a monospecific anti-PSA antiserum was obtained. Subsequently, more antisera were prepared to ascertain its specificity. Indeed, by the conventional immunodiffusion or IEP, the antisera were shown to be reactive only with human prostate.

The first generation antiserum specific for PSA was used for at least two purposes: (a) in the construction of semi-quantitation of PSA for the measurement of PSA from the prostate tissue extracts; and (b) more importantly, in monitoring the isolation of PSA from prostate tissues. As soon as the antiserum specific for PSA was generated, Dr. Wang undertook the purification of PSA. The initial purification protocol of PSA involved extraction with disodium ethylene-diamine tetracetate(EDTA)-phosphate buffered saline,

fractionation with ammonium sulfate (35-55%), ion exchange chromatography on diethylaminoethyl (DEAE)-BioGel, and gel filtrations on Sephadex G-100 and G-75, followed by a final step of preparative polyacrylamide gel electrophoresis (PAGE)--a difficult technique even today. This complex procedure produced a low yield of purified PSA. A simplified purification was developed thereafter by eliminating Sephadex G-75 and preparative PAGE steps, and with an additional DEAE step, which resulted in a 3-fold greater yield of purified PSA (2).

The homogeneity of the purified PSA preparation was indicated by a single protein band with no contaminating components in sodium dodecyl sulfate (SDS)-PAGE in the presence or absence of reducing agent, and by isoelectric focusing that revealed a single isoelectric point of 6.9. Furthermore, the purified PSA was shown to have a molecular weight of 33,000 by Sephadex 75 and 34,000 by SDS-PAGE, with no subunit components.

The 1979 paper (1) suggested the potential significance of PSA in clinical application, as initial results indicated that this prostate antigen, although a eutopic component of the prostate, may play a role in the detection of prostate cancer. Thus, attention was turned to the development of the PSA blood test.

PSA BLOOD TEST

Shortly after the generation of specific anti-PSA antiserum, Dr. Lawrence D. Papsidero, at the time a recently graduated Ph.D. student, used the anti-PSA antiserum to identify the presence of PSA in the sera of metastatic prostate cancer patients by rocket-IEP (3). Further, PSA obtained from patients' sera and from prostate tissues were demonstrated to be immunologically identical. These significant findings provided the basis for the development of an enzyme-linked immunosorbent assay (ELISA) a few months later by Dr. Manabu Kuriyama, then a research fellow.

Noteworthy at that time, and even more so today, was Dr. Papsidero's observation that when sera from metastatic prostate cancer patients were examined by gel filtration, the reactive antigen, or circulating PSA, eluted as a peak at a molecular weight of 96,000, while the antigen isolated from prostate tissues eluted at 34,000. Additional studies with immunoprecipitation and two-dimensional IEP revealed that, in addition to PSA, the serum PSA peak contained "normal serum protein contaminants." This was the first report concerning complexed PSA, as it is called today.

The data published in the initial *Cancer Research* article that resulted from this work provided new insight into the biology of prostatic tumors, and demonstrated that prostate specific proteins, such as PSA, are released from prostate cells during the course of tumor development. In addition, the results prompted the development of a more sensitive assay for PSA in order to detect prostate cancer at earlier stages.

Upon receiving the acceptance notification from *Cancer Research* in mid-April of 1980, the author immediately submitted a follow-up paper describing the quantitation of PSA in serum by ELISA (4). Essentially, the test was a traditional sandwich-type ELISA, in which all reagents had been "home-made" by Dr. Kuriyama and others in the lab, including purified PSA, monospecific polyclonal anti-PSA, solid-phase conjugated anti-PSA IgG, and indicator anti-PSA conjugated to horseradish peroxidase. This paper reported to the world the first sensitive, reproducible, and reliable quantitative assay for serum PSA. The sensitivity of this ELISA was 0.1 ng/ml, equivalent to that of the PSA assays commonly available today.

The ELISA confirmed the previous IEP findings that only tissues of prostate origin contained significant amounts of PSA, and that no statistically significant difference in PSA levels was found among normal, benign, and malignant prostates. However, the ELISA results were most dramatic in the serum test, which revealed that no PSA was detected in female patients with various cancers and that PSA levels in male patients with non-prostate cancers were similar to those of normal male controls. Importantly, the serum

ELISA established that PSA values in the blood of patients with BPH and prostate cancer were significantly greater than those of controls. In addition, no significant difference was found between PSA values in BPH and in stage A prostate cancer, a suggestive difference was found between BPH and stage B, and a significantly higher PSA level was found in stage C and D diseases than that in BPH. Further, it was encouraging that PSA was a better disease parameter than PAP, which was at that time the "gold-standard" for monitoring prostate cancer (5,6). These results clearly suggested for the first time the utility of PSA in diagnosis of prostate cancer.

Through the National Cancer Institute (NCI)-sponsored National Prostatic Cancer Project (NPCP), the potential clinical application of PSA was evaluated in a double blind manner (7). Great acknowledgment is due to the contribution of Dr. G.P. Murphy, the NPCP Director, as well as to the many colleagues of urologists involved with the NPCP, without whom the clinical significance of PSA would not have been so soon realized: Dr. S. Loening (Iowa City),
Dr. R.P. Gibbons (Seattle), Dr. G.R. Prout (Boston), Dr. W.W. Scott (Baltimore), Dr. M. Soloway (Memphis), Dr. J.D. Schmidt (San Diego), Dr. S. Bergman (New Orleans), Drs. J.E. Pontes and J.M. Pierce (Detroit), Dr. P. Scardino (Houston), Dr. D. Mcleod (Washington, D.C.), Dr. C. McKiel (Chicago), Dr. J. deKernion (Los Angeles), and Dr. S. Beckley (Buffalo). The prognostic value of PSA was detected immediately from this study. PSA levels prior to treatment were shown to be correlated with survival rate in the evaluation of patients with metastatic prostate cancer, regardless of the treatment regimen received by the patients. Serial PSA tests were shown to be of significant value in monitoring prostate cancer. Using the patient as his own reference, PSA levels increased as disease progressed, decreased as disease regressed, and remained fluctuating when the patient was in stable condition.

An additional significant observation was made early in the clinicopathological evaluation of PSA. Patients with localized prostate cancer who underwent curative therapy and later developed metastasis during the follow-up period were found to exhibit increasingly elevated PSA before disease recurrence was detected clinically. For example, in patients who received radical prostatectomy, the average lead-time between elevation of PSA and clinical detection of disease recurrence was shown to be approximately 12 months. Therefore, by 1981, the monitoring capability of PSA was fully appreciated.

By 1983, Dr. Wang and colleagues made the first step in the biochemical characterization of PSA, revealing a glycoprotein consisting of approximately 7% carbohydrate and 93% peptide (8). Considering that PSA represents 0.1% of total protein extractable from the prostate, and is secreted into the prostatic fluid in exceedingly high concentrations of up to 3.6 mg/ml, the question arose as to its biological function, if any. The response was published in 1984 by Dr. Yoshihito Ban, then a research fellow, in the first paper reporting the protease activity of PSA (9). PSA is a unique protease, as its substrate specificity is different from those of known proteases. Initial investigation of substrate specificity, inhibitor specificity (9), and later the complete amino acid sequence reported by Dr. Rueyming Loor (10), a former colleague, led to the conclusion that PSA is a chymotrypsin-like serine protease. It should be noted that the demonstration of PSA as a chymotrypsin-like serine protease, and of the molecular weight of circulating PSA predominately as 96,000 instead of 34,000 as in prostate tissue, served as the genesis of today's form of PSA complexed to antichymotrypsin of molecular weight 69,000.

PSA TISSUE TEST

In addition to the PSA blood test, the author and coworkers also developed a PSA tissue test. It has been well recognized that the clinical application of PSA is based upon the prostate specificity of the PSA molecule (1,11,12). Prostate tissue specificity and cell-type specificity of PSA was well appreciated by 1981, as is well documented in several papers (1,13-15). In January 1981, the PSA-specific distribution of human prostate ductal epithelial cells was presented in detail (13). Soon thereafter, Dr. Mehrdad Nadj, a colleague and pathologist at the University of Miami, reported for the first time that PSA is an immunohistopathological

marker for prostate cancer (15). Its use is especially effective in the identification of distant metastatic prostate carcinoma, and in the differential diagnosis of poorly-differentiated transitional cell carcinomas of the bladder (negative immunohistochemistry) from prostate carcinoma.

PSA IN SCREENING

At present, the most important clinical use of PSA is as an aid in the screening of high-risk and older populations for early detection of prostate cancer. Several groups of urologists and investigators were principally responsible for the establishment of PSA in screening use, including Cooner et al. in 1988 (16), Catalona et al. in 1991 (17), and Brawer et al. in 1992 (18). Primary among these was Catalona's group, who reported in 1991 the first large series of investigations examining the role of PSA as a screening tool (17). In response to a press release seeking participation of men 50 years of age or older in a study of PSA as a screening test for prostate cancer, 1,653 healthy asymptomatic men volunteered. Thirty-seven men, or 2.2%, were diagnosed as having cancer. Had digital rectal examination (DRE), the traditional screening test, been used alone without PSA, 32% of the cancer identified would have been missed, suggesting the superiority of PSA over DRE alone.

Two years later, in 1993, Catalona et al. further reported in an expanded study that PSA screening is effective in the detection of organ-confined, early-stage prostate cancer (19). This time the PSA study group consisted of 10,251 healthy asymptomatic men and was divided into two subgroups: the initial PSA-based screening group and the serial PSA-based screening group. The control comparison group consisted of 266 men of the same age range who were referred to the clinic because of an abnormal DRE. Among prostate cancers detected, the proportion that were
organ-confined early-stage were 63%, 71%, and 43%, respectively, for the initial PSA-based screening group, serial PSA-based group, and DRE comparison group. These results indicated that men 50 years of age or older who were examined by a simple PSA blood test and found to have prostate cancer were more likely to have early cancers, especially if they were 70 years of age or younger, than were men examined by traditional DRE only. Similar results have been reported from Japan (20). This study clearly demonstrated that PSA-based screening is effective in the detection of early prostate cancer, and suggested that men aged 70 or younger who undergo a PSA screening are more likely to benefit from early detection, as they have a longer life expectancy.

The American Cancer Society (ACS)-sponsored National Prostate Cancer Detection Project reported similar findings to those described above, supporting the value of PSA in detecting early prostate cancer (21). The ACS in late 1992 finally recommended the use of PSA and DRE in the annual physical examination for men 50 years or older (22). According to ACS recommendations, high-risk men such as African-Americans and men who have first-degree relatives with prostate cancer are urged to commence annual screening at age 40. It is generally agreed that asymptomatic men who have both negative DRE and normal PSA blood test need only to continue an annual PSA and DRE check-up. Men who have a negative DRE and elevated PSA, and all those who have a suspicious DRE regardless of PSA results, should undergo further diagnostic workup, such as transrectal ultrasonography with biopsy of visible lesions. The American Urologic Association and the American Association of Radiology have recommended the same guideline.

An apparent consequence of PSA screening is the rising incidence of prostate cancer in recent years. The incidence of prostate cancer is now surpassing that of lung cancer in men in the United States. According to the 1993 data from the NCI Surveillance Epidemiology and End Results (SEER) program, detection of prostate cancer increased at an unprecedented rate of 16% between 1989 and 1990 and 30% between 1990 and 1991. The greatest increase was in stage A and B localized cancers, supporting the widely-held belief that PSA detection of tumors in asymptomatic men is responsible for the increase. Indeed, two recently published papers have confirmed this suggestion. One report revealed that the recent apparent epidemic of prostate cancer is due in part to the increasing detection of tumors resulting from increased PSA screening (23). The

increase was predominantly for organ-confined tumors. The other report suggested that the increased incidence appears to be a transient phenomenon due to the depletion of previously undiagnosed cases from the prevalence pool (24).

PSA screening for prostate cancer and staging workup of disease appear to have produced an acceptable morbidity (19). To the extent that the high prostate cancer mortality rate is primarily due to late detection, and the treatment of early cancer is generally effective, PSA-based screening should logically reduce the prostate cancer mortality rate (19,25). Yet, in terms of potential use of PSA in screening, the issue of morbidity is more difficult. Most patients in whom an elevated PSA is found subsequently undergo ultrasound-guided biopsy. However, serum PSA testing has not been accepted universally for routine screening, as it may involve some unnecessary biopsies. Furthermore, for those individuals in whom an elevated PSA is followed by a positive biopsy result, a difficulty exists in discerning whether histological evidence of prostate cancer will manifest clinically in morbidity and mortality during the patient's lifetime.

Based on the complexity of these issues, a heated debate has been underway regarding whether PSA is indicated as a screening test. The controversy is epitomized by the discrepancies in the recommendations given by various institutions in various countries. The Canadian Task Force on the Periodic Health Exam does not recommend routine PSA screening in asymptomatic males over 50 years of age; the American and Canadian Urological Associations promote annual screening beginning at age 50. Somewhere in the middle is the National Cancer Institute, which concluded in 1994 that there is insufficient evidence at present to determine whether serum PSA should be recommended for routine screening.

Significant questions remain as to whether detection of early-stage organ-confined disease will finally increase the survival rate of patients, and whether the expenses of workup and treatment of early prostate cancer are justified (26). To answer these questions, the NCI four years ago launched a multicenter, 16-year, large-scale follow-up of over 37,000 men aged 60 to 74 years (27). The Department of Veteran's Affairs (VA) has also launched a similar trial to enroll 2,000 participants, with follow-up of 15 years (28).

CONCLUSION

By 1981, the occurrence of PSA in human prostate and prostatic fluid had been shown, in vitro and in vivo expression of PSA by prostate tumor proven, PSA purification from the prostate and seminal plasma and subsequent characterization achieved, PSA occurrence in serum documented, ELISA of serum PSA developed, and diagnostic and prognostic potential of PSA in prostate cancer demonstrated (31,32). The value of PSA as a new prostate cancer marker had been established. Since 1981, several biomedical manufacturers have made the PSA blood test and tissue test widely available. In 1986, PSA was approved by the U.S. Food and Drug Administration for use as a monitor for treatment response and disease recurrence of prostate cancer, and in 1994 as an aid for early detection (i.e., screening) of prostate cancer.

Drawing an analogy between prostate cancer in men and breast cancer in women, the eminent urologist Dr. Patrick Walsh has asserted:

> In the United States a new diagnosis of prostate cancer is made every 3 minutes and a man dies of the disease every 15 minutes--25% of men with prostate cancer [still] die of it, similar to the percent of women with breast cancer who die of it...Although there are many critics of PSA screening, there is no question that it is more effective than mammography (33).

At present, a debate surrounding PSA screening rages on; yet, the debate is not about PSA itself, but rather concerns treatment. Particularly at issue are the options, efficacy, complications, and cost of treatment. Paradoxically, although PSA screening identifies early prostate cancer, the urologists/oncologists and the patients are left with the difficult decision of how to treat it: by surgery, radiation, or watchful waiting (30).

Hopefully, in the
not-too-distant future, all these issues can be resolved.

If even one man's life is saved by the PSA test, the work in PSA has been a success.

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