Evaluation of a Monoclonal Immunoradiometric Assay for Prostate-Specific Antigen

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We evaluated the analytical performance of a new monoclonal immunoradiometric assay ("M-PSA") for prostate-specific antigen ("Tandem"; Hybritech Inc.) in comparison with a monoclonal immunoradiometric assay ("M-PAP") for mass measurement of prostatic acid phosphatase ("Tandem") and with a conventional enzyme-activity assay ("E-PAP") for prostatic acid phosphatase (EC 3.1.3.2). For M-PSA, the CVs were 1.3–3.0% within-run and 3.0–4.9% between-run. The minimum detectable mass concentration was 0.10 μg/L, and linearity extended to 100 μg/L. The reference interval for M-PSA in 178 healthy men was 0–2.8 μg/L. Serum specimens from men with prostatic disease (primarily prostatic carcinoma and benign prostatic hypertrophy) were assayed by the three methods. Correlation was best between mass measurement (M-PAP) and enzyme activity (E-PAP) for prostatic acid phosphatase (r = 0.958). Results for PSA did not correlate well with those for either M-PAP (r = 0.629) or E-PAP (r = 0.387). PSA was increased in a higher percentage of specimens from men with earlier (clinical stage B) prostatic carcinoma than were results from either assay for PAP.

Additional Keyphrases: prostatic carcinoma · enzyme-activity and mass techniques compared · reference interval · cutoff values · benign prostatic hypertrophy

Adenocarcinoma of the prostate is the most common neoplasm in men older than 70 years, and is the third most prevalent neoplastic cause of death for all men (1). Unfortunately, many prostatic cancers have already spread beyond the limits of curative surgery at the time of initial detection.

Various laboratory assays for different serum constituents have been used in efforts to diagnose prostatic cancer at earlier, potentially curable stages, and their analytical and clinical performance has been reviewed (2–5). Such assays have included measurement of enzymes such as acid phosphatase (either total or the fraction thought to be derived from the prostate), alkaline phosphatase, creatine kinase-BB, and isoenzymes of lactate dehydrogenase, as well as other non-enzymatic protein tumor "markers" such as carcinoembryonic antigen. Assay of the activity of the prostatic fraction of acid phosphatase (PAP; EC 3.1.3.2) in serum is currently the most widely used laboratory test in diagnosis and management of prostatic cancer. Although extensive efforts have been made to improve the clinical performance of PAP assays, either by modifying the reagents in assays of enzymatic activity or by developing immunoassays to measure the mass concentration of PAP, these efforts have not allowed earlier detection of the disease in a substantial proportion of patients tested (2).

Recently, a new prostate-specific protein antigen (PSA) has been isolated and identified from prostatic epithelial cells and detected in sera from patients with prostatic cancer (6). PSA is physically, chemically, and immunologically distinct from prostatic acid phosphatase. It is a glycoprotein with a molecular mass of approximately 33 kDa. Normal, hyperplastic, and neoplastic prostatic tissues contain similar concentrations of PSA. It is not detectable (with current assays) in serum from women. Serum from patients with advanced prostatic cancer consistently contain increased concentrations of PSA as compared with serum from normal men (7).

PSA is highly immunogenic, and immunoassays have been developed involving either polyclonal antibodies (7–9) or monoclonal antibodies (10–12) with use of either radioisotopes or enzymes as labels for either antigen or antibody.

Here we compare two immunoradiometric assays in which monoclonal antibodies are used—one for PAP (M-PAP) and the other for PSA—with a conventional assay for PAP based upon catalytic activity (E-PAP) in normal subjects and in men with a variety of prostatic diseases (primarily benign prostatic hyperplasia and prostatic adenocarcinoma). We included patients with earlier stages (A and B) of prostatic cancer, who were candidates for radical prostatectomy, as study subjects for detailed evaluation of assay performance—both for efficiency of detection as well as effectiveness of monitoring for recurrence of disease.

Materials and Methods

Patient populations. All patients were seen at The Johns Hopkins Hospital and its ambulatory-care facilities. The total study population consisted of 128 men with prostatic disease who were seen by the professional staff of the Department of Urology (which is a referral center for patients with prostatic cancer in earlier stages who are candidates for radical prostatectomy). A reference population of 269 subjects consisted of 91 women and 178 men. Clinical history and histological diagnosis were available for all patients. Portions of the data in this report were previously presented at the 1986 national meeting of the AACC (Clin Chem 1986;32:1125). Received July 9, 1987; accepted September 9, 1987.
all 128 men with prostatic disease. The clinical stage of prostatic carcinoma was established independently of the PSA concentration in serum, according to the criteria of the American Urological Association.

Specimens were collected into evacuated tubes without anticoagulants. Serum was separated and aliquots were stored at −20 °C until analysis.

Assay for PSA. PSA in serum was measured by using a solid-phase, double-antibody immunoradiometric assay ("Tandem™-R PSA"; Hybritech, Inc., La Jolla, CA 92212). A 50-μL sample was incubated simultaneously with one monoclonal antibody to PSA coated on a plastic bead and 200 μL of a solution containing another 125I-labeled monoclonal antibody (directed at a separate and distinct antigenic site on the PSA molecule). After a 2-h incubation at room temperature, the plastic bead and tube were washed with a solution containing detergent and sodium azide (3 g/L). Radioactivity bound to the plastic bead was then counted in a gamma counter.

Calibrators over the concentration range of 0 to 100 μg of PSA per liter, as well as control materials, were supplied by the manufacturer. The entire assay sequence was performed in accordance with the manufacturer's written instructions.

Immunoradiometric assay for PAP. PAP in serum was measured by using a solid-phase, double-antibody immunoradiometric assay involving two monoclonal antibodies directed at different antigenic sites on the PAP molecule. (Tandem™-R PAP; Hybritech, Inc.) (13). The entire assay sequence was performed in accordance with the manufacturer's written instructions. The stated reference interval for healthy men was 0–3.0 μg/L (97.5th percentile).

Enzymatic assay for PAP. Activity of PAP in serum was measured at 37 °C, with sodium thymolphthalein mono-phosphate (Worthington Phosphatase Reagent Kit, cat no. 27397; Cooper Biomedical, Inc., Malvern, PA 19355) as substrate (14). The reference interval specified by the supplier for this assay in healthy men is 0.8 U/L (97.5 percentile).

Results

Precision. Table 1 shows the precision of the assays for PSA and PAP.

Linearity. The M-PSA assay curve was linear over the working range of 0–100 μg/L, as established by serial dilution of patients' samples with the "zero" calibrator material. Analytical recoveries (percent of expected PSA result) ranged from 97% to 102%.

Analytical sensitivity. The minimum detectable concentration for M-PSA was 0.10 μg/L (calculated as the mean of 20 replicate determinations of the zero calibrator plus 3 SD).

Reference interval for M-PSA. For sera from 91 healthy women, the M-PSA concentration in serum was <0.2 μg/L (97.5th percentile). For 92 healthy men below the age of 40, the M-PSA concentration in serum was <2.0 μg/L (99th percentile). For 86 healthy men above 40 years of age, the M-PSA concentration was ≤2.8 μg/L (99th percentile).

Table 1. Precision of Assays of PSA

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Analytical comparison of methods. Figure 1 (A–C) illustrates the correlation of results by the three assays. The best correlation was between M-PAP and E-PAP (Figure 1C; r = 0.9854). M-PSA did not correlate well with either M-PAP (r = 0.6285, Figure 1A) or E-PAP (r = 0.5986, Figure 1B).

Clinical comparison of methods. Figure 2 (A–C) shows results for M-PSA, M-PAP, and E-PAP in patients with the four major stages A–D of prostatic cancer, as well as in patients with nodular hyperplasia of the prostate (clinically termed "benign prostatic hypertrophy," or BPH). All patients with stages C and D of prostate cancer had abnormally high M-PSA concentrations in their serum; serum M-PAP and E-PAP were within the reference intervals in some of these patients. A significant percentage (83%, 40/48) of patients with stage B prostate cancer had increased serum M-PSA concentrations (using 2.8 μg/L as a decision level) with M-PAP and E-PAP results within their respective reference intervals. Although some patients (4/11, or 36%) with early stage-A prostate cancer had increased serum M-PSA concentrations, the majority did not. M-PAP and E-PAP results in this group of patients were within the reference interval. Approximately half (19 of 37) of patients with benign prostatic hypertrophy, however, had increased concentrations of PSA in serum but results for serum M-PAP and E-PAP that were within the reference intervals.

Table 2 lists clinical sensitivity and specificity (15) for correct identification of all patients with prostatic cancer, for each of the three tests. Although the sensitivity of M-PSA was much higher than that of M-PAP and E-PAP, the specificity of M-PSA was lower than that for the tests for PAP, primarily because of the overlap of increased M-PSA concentrations in serum between results for patients with prostatic cancer and those with benign prostatic hypertrophy.

Discussion

A two-site immunoradiometric assay for serum prostate-specific antigen involving monoclonal antibodies performed well analytically, in terms of precision, linearity, and minimum detectable concentration.

The minimum detectable concentration observed in this study was 0.10 μg/L, which is consistent with a value of 0.10 μg/L obtained in early studies by enzyme immunoassay (7, 8), and it is to be compared with 0.25 μg/L in a study

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3 The clinical system adopted by the American Urological Association for the classification of prostatic carcinoma of the prostate consists of the following stages: A: incidental finding at prostatic surgery (A1: focal; A2: diffuse); B: confined to prostate by digital rectal examination (B1: small discrete nodule; B2: multiple nodules); C: localized to periprostatic area (C1: prostate <70 g, seminal vesicles uninvolved; C2: prostate >70 g, seminal vesicles involved); D: metastatic disease (D1: metastases to pelvic lymph nodes or ureteral obstruction; D2: distant metastases to other sites).
evaluating a commercially available radioimmunoassay (9) and 0.4 μg/L with an enzymoimmunometric assay (11). Analytical sensitivity at low concentrations of antigen is important in monitoring patients for recurrence of tumor, particularly in patients who have undergone total prostatectomy and should have no residual source of PSA.

We observed that all serum M-PSA values for women were below 0.2 μg/L, whereas healthy younger men (below age 40) had appreciably higher serum M-PSA concentra-

tions, with the 99th percentile at 2.0 μg/L. Of the 91 women in our study, only two had values >0.11 μg/L. Other studies (7, 9) have found M-PSA concentrations in serum from women to be at or near the limits of detection. Because the values, if any, measurable in women are probably artificial, these findings are consistent with the localization of PSA to prostatic tissue.

Although PSA is specific for prostatic tissue, the antigen
can be identified in samples of tissue from normal prostate and from prostate in cases of benign prostatic hyperplasia and adenocarcinoma of the prostate (6). The prevalence of both benign hyperplasia and adenocarcinoma of the prostate increases after the fifth decade of life, and the two conditions may occur together in the same patient.

Comparison of the upper limits of the reference interval, the cutoff points, or the decision levels for PSA used in different studies to classify patients is complicated by the lack of a well-defined analytical standard material that could be used to evaluate the calibration differences among different immunoassays for PSA. Decision levels used to classify PSA results as increased have ranged from 1.8 μg/L (7) to 2.5 μg/L (8) to 2.7 μg/L (9) to 4.0 μg/L (11). Incorporation of "healthy" older men into the reference population may increase the number of subjects with subclinical or mildly symptomatic benign prostatic hypertrophy.

Selecting a decision level for PSA concentrations in serum that will allow clinically accurate discrimination between benign prostatic hypertrophy and prostatic carcinoma has proved difficult. Kuriyama et al. (7), using a decision level of 1.8 μg/L, found increased PSA in 68% of sera from subjects with BPH, compared with increased PSA in 63–77% of sera from patients with stages A–C of prostatic carcinoma. In contrast, Takeuchi et al. (8) found serum PSA concentrations above their selected decision level of 2.5 μg/L in only 7% of patients with BPH. Using a radioimmunoassay for PSA and a decision level of 2.7 μg/L, Liedtke and Batjer (9) noted increased PSA concentrations in 86% of sera from patients with BPH. (In the present study, we found 60% of BPH patients to have M-PSA concentrations above our decision level of 2.8 μg/L.) Considering the high prevalence of BPH in older men, the finding of increased serum PSA concentration in this age group is not specific for prostatic carcinoma.

The effects on clinical sensitivity and specificity of selecting differing decision levels are illustrated in Figure 3, with receiver operating characteristic curves shown for PSA, M-PAP, and E-PAP. As the performance of a test improves, this curve (as plotted in Figure 3) will shift upwards and to the left as true-positive rates increase and false-positive rates decrease (16). Our data for the three assays performed on our study patients, so plotted, indicate that the assay for PSA performs "better" in the above sense than does either assay for PAP. The PSA assay, however, shows no clear change in the slope of the receiver operating characteristic curve at any specific decision level, unlike the receiver operating characteristic curves for M-PAP and E-PAP, which show such changes at decision levels of 3.0 μg/L and 0.8 U/L, respectively. We selected a decision level of 2.8 μg/L PSA for our analysis of data, recognizing that at that level a substantial portion of patients having BPH would be classified as having increased serum PSA concentrations (false positives).

Serum PSA concentrations are increased above the upper limits of assay reference intervals for most patients with prostatic carcinoma (7–9), with greater increases noted for patients with more extensive disease (stages C and D). Our data showed M-PSA >2.8 μg/L for the following clinical stages of prostatic cancer: stage A, 4/11 (36%); stage B, 40/48 (83%); stage C, 10/10 (100%); and stage D, 21/21 (100%). As Figure 2 indicates, we did find progressive increases in serum PSA concentration with successive stages A–D of cancer.

We also found M-PSA to be clinically more sensitive than

![Fig. 3. Receiver-operating characteristic (ROC) curves for PSA, M-PAP, and PAP](image-url)

The data for all 128 patients with prostatic disease are plotted, with several quantitative decision levels (as indicated in the figure) for each assay. Units are μg/L for M-PAP and PSA, and U/L for E-PAP.

M-PAP and E-PAP, with a higher proportion of prostatic cancer patients having increased M-PSA concentrations than increases in M-PAP or E-PAP (Figure 2). PAP measurements, even by several different techniques, have not had the requisite sensitivity to be used in screening or in early diagnosis (17). M-PSA has a different problem of specificity: that caused by the overlap between results for patients with early stages of prostatic cancer and those for patients with BPH.

Because PSA is produced exclusively by the prostate, the decreasing concentration of PSA in serum after radical prostatectomy can be used to determine the presence of residual prostate tissue. Serum PSA higher than the predicted postoperative value correlates with subsequent recurrence of disease (18).

Measurement of PSA in serum is also useful in monitoring patients with previously diagnosed prostatic carcinoma for recurrence of disease. Killian et al. (19) found increased serum PSA concentrations in 24 of 26 patients an average of 12 months before recurrence was noted clinically. In a subsequent study (20), these same authors reported PSA to be more sensitive than other serum measured markers (serum acid phosphatase, either prostatic or total, total alkaline phosphatase in serum, or the bone-derived isoenzyme of alkaline phosphatase in serum) in detecting early recurrence of prostatic cancer.

In addition to PSA, other protein markers have been immunologically identified that appear to be localized to the prostate. One of these, γ-semioprotein, was originally identified in normal seminal plasma for forensic purposes (21), and an enzyme immunoassay involving monoclonal antibodies to this antigen has been studied with sera from patients with prostatic cancer (22). PSA and γ-semioprotein show some immunochemical similarities, and the two antigens both may be useful markers for progression of prostatic carcinoma (23). At least two other prostate-specific antigens have been described (24, 25), which can be recognized immunochemically in vivo or in tissue culture.
These antigens, however, are also found in normal as well as malignant tissue (as is PSA), and no published reports evaluating their use as serum markers for prostatic carcinoma are as yet available.

In summary: we find a two-site immunoangiometric assay in which monoclonal antibodies to prostate-specific antigen (M-PSA) are used has good analytical precision, linearity, and sensitivity (limit of detection). Initial clinical evaluation indicated that serum M-PSA was more frequently increased in the earlier stages of prostatic carcinoma than were results of two different assays for PAP, one a traditional assay based upon enzyme activity and the other an immunoangiometric assay. Concentrations of PSA in serum of patients with benign prostatic hypertrophy appear to overlap those of patients with the earliest stage A of prostatic carcinoma, and the assay for M-PSA is not sufficiently specific clinically to use as a screening test for the diagnosis of prostatic cancer in its earliest asymptomatic stage. The clinical progression of prostatic cancer, however, was associated with increasing concentrations of M-PSA in serum. The assay may be most useful in monitoring patients for presence of residual disease after radical prostatectomy and for recurrence of disease after treatment.

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References