Comparison of Enzyme-Linked Immunosorbent Assay and Radioimmunoassay for Prostate-Specific Acid Phosphatase in Prostatic Disease

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We compared results by an enzyme-linked immunosorbent assay (ELISA) with those by a standard radioimmunoassay (RIA) for detection and quantitation of prostate-specific acid phosphatase (EC 3.1.3.2) in serum. Control subjects, patients with benign prostatic hyperplasia, and patients in all four clinical stages of prostatic adenocarcinoma were tested. The upper limit of normal (95% of the population) by the ELISA was 2.0 µg/L, and by the RIA was 2.2 µg/L. In prostatic adenocarcinoma stage I (not detectable by digital rectal examination), ELISA was slightly more sensitive than RIA, but sensitivity was still relatively low (20%). As tumor mass increased (stages II through IV), the frequency of increased concentrations of prostate acid phosphatase in serum also increased. We confirmed this increase in circulating enzyme in some cases of benign prostatic hyperplasia and suggest that this finding is related to either acinar cytolysis or an increase in acini size and number. Although prostate-specific acid phosphatase is not a cancer-specific enzyme, we conclude that its measurement may be of considerable value in monitoring prostatic disease.

Additional Keyphrases: cancer • benign prostatic hyperplasia • adenocarcinoma • cutoff value • isoenzymes

Increases of prostate-specific acid phosphatase (orthophosphoric monoester phosphohydrolase, acid optimum; EC 3.1.3.2; PSAP)³ have been used in conjunction with digital examination of the rectum for detection of prostatic adenocarcinoma (1). In clinical stages II, III, and IV, where the tumor can be palpated (2), the value of increased enzyme activity is merely prognostic, serving as a "baseline" indication for the success or failure of treatment. However, in stage I, where by definition the examining surgeon cannot so detect the presence of a tumor, the finding of serum PSAP values exceeding the calculated reference interval for healthy individuals should prompt further examination. At this stage, assay specificity, detection limit, and reproducibility in the lower range are extremely important, because the few active neoplastic cells will be secreting only small amounts of the enzyme into the circulation. In particular, it is important to assure that only the prostate-specific isoenzyme is being measured.

Several RIA methods for PSAP are now commercially available. However, one disturbing feature in comparative kit assessment has been the wide range of "normal" values, especially at the upper limit, which may vary from 1.9 to 7.15 µg/L with different kits (3–5). The health-associated reference interval for PSAP as measured immunologically remains to be defined. Alternative methods for immunological quantitation of enzyme mass—enzyme-linked immunosorbent assay (ELISA) (6) and solid-phase fluorescent immunoassay (7)—have recently become available and thus permit a practical comparison with RIA.

We evaluated a recently developed ELISA system for PSAP, in terms of cutoff values for men over 40 years old, detection limit, and specificity of the assay. We also investigated the ELISA's performance with regard to untreated patients with benign prostate hyperplasia or prostatic adenocarcinoma (at all four clinical stages), and compared these results with those by a RIA.

Materials and Methods

Laboratory Evaluation of PSAP

Enzyme-linked immunosorbent Assay (ELISA). For this we used "Enzygnost" PSAP kits (Calbiochem-Behring Corp., La Jolla, CA 92037), based on a solid-phase double-antibody "sandwich" assay (6). PSAP purified from human seminal fluid by sequential gel filtration, ion-exchange chromatography, and preparative isoelectric focusing was used to immunize rabbits and sheep. Antibodies from sheep were purified by affinity chromatography and used to coat polystyrene reaction tubes. Affinity-purified antibody from rabbit was conjugated to horseradish peroxidase (EC 1.11.1.7) to serve as the enzyme tag. Human serum depleted of PSAP by immunosorption was reconstituted with purified PSAP to provide standards.

To perform the assay, add patients' sera or standards (100 µL) to tubes containing 100 µL of a protein-supplemented medium for incubation. After incubation for 2 h at room temperature, aspirate the liquid and wash the tubes twice with a buffer. Add peroxidase-labeled anti-PSAP conjugate to react with the specifically retained PSAP during a second 2-h incubation. After a second washing, measure the enzymic activity of the retained conjugate by adding the chromogenic substrate (o-phenylenediaminedihydrochloride) and hydrogen peroxide. After a 30-min incubation, terminate the reaction by adding 0.25 mol/L H₂SO₄, and measure the absorbance at 492 nm. We used a Model 300 N spectrophotometer (Gilford Instrument Laboratories, Oberlin, OH 44074). Figure 1 shows a typical standard curve.

We determined the detection limit for this method, defined as the concentration corresponding to the mean plus 2.6 standard deviations of the signal of a zero standard (8), to be less than 0.1 µg/L. To test linear relation to concentration, we diluted a sample containing about 50 µg of PSAP per liter five-, 10-, 20-, and 40-fold with incubation medium and assayed these dilutions. A plot of dilution factor vs concentration detected yielded a straight line.

The antibodies used were specific for acid phosphatase of prostatic origin. Lyed platelets, erythrocytes, or leukocytes, added to PSAP-containing serum, gave no cross reactivity for PSAP in the ELISA.
Radioimmunoassay. The RIA has been described in detail elsewhere (5). We also used Prostatic Acid Phosphatase RIA kits from New England Nuclear, Boston, MA 02118, in accordance with instructions in the manufacturer's insert. Both RIA and ELISA kits involve use of PSAP extracted from human seminal fluid by similar methods for preparation of standards and for antibody production.

Studies of Patients

The patients we studied were 40 to 87 years old. Blood was sampled at least 24 h after rectal examination, stored in aliquots as nonacidified serum at −70 °C, and tested in batches. Materials were thawed only once, tested, and then discarded. Diagnoses of benign prostatic hyperplasia and adenocarcinoma were confirmed by histological section of prostate tissue. The clinical stage of adenocarcinoma cases by the criteria of Whitmore (2) was established by rectal digital examination, prostatic surgery, bone-marrow examination, pelvic lymphadenectomy, and bone scan. Stage I adenocarcinoma is defined by a positive biopsy, with the tumor not detectable by palpation. Stage II adenocarcinoma is characterized by a palpable tumor, limited within the prostatic capsule. Stage III is defined by a palpable tumor in the prostate and seminal vesicles. In stage IV, the adenocarcinoma is disseminated. The tumor mass in cases of benign prostatic hyperplasia was estimated at the time of rectal examination by the urological surgeon.

Patients with no symptoms of urogenital disease, but hospitalized or visiting a clinic for other complaints, were used as the comparison population.

Serum samples were assayed without knowledge of the results of the clinical staging of patients.

Results

Method comparison. Results of the two immunoassay methods for 99 concurrently tested patients' samples were compared by simple linear regression (Figure 2). The slope of the line was 0.95, the y-intercept 0.3 µg/L, and the coefficient of correlation (r) was 0.95. In a paired t-test the t-value was 0.03, indicating a high degree of correlation between the two tests.

Cutoff values. Figure 3 shows the distribution of serum PSAP concentrations as measured by ELISA and RIA in 97 patients clinically free of prostatic disease. The range of values for each extended from less than the lowest standard to 2.7 µg/L for ELISA or 3.0 µg/L for RIA.

We chose as the upper limit of normal that concentration at or below which 95% of the values for the prostatic-disease-free persons fall. For 97 sera from the comparison group tested by RIA, the cutoff point was 2.2 µg/L. Thus, any PSAP concentration exceeding 2.2 µg/L was defined as "elevated." For the same 97 sera tested by ELISA, the cutoff point was 2.0 µg/L.

Detection of prostatic disorders. Having established a cutoff value for each assay method, we applied them to results from patients known to have either prostatic cancer or benign prostatic hyperplasia (Table 1). For the patients with benign prostatic hyperplasia, both tests designated 20% of them as PSAP-elevated. For staged prostatic cancer cases, two trends are evident. As the tumor stage increases, it is more likely to be accompanied by increased serum PSAP. Also, the ELISA
Table 1. Comparative Diagnostic Accuracy of Three Methods

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. of patients</th>
<th>No. samples with above-normal PSAP a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign prostate</td>
<td>84</td>
<td>RIA b (17) (20%) ELISA c (17) (20%) RIA d (9%)</td>
</tr>
<tr>
<td>hyperplasia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostatic cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>25</td>
<td>4 (18%) 6 (24%)</td>
</tr>
<tr>
<td>Stage II</td>
<td>25</td>
<td>10 (40%) 11 (44%)</td>
</tr>
<tr>
<td>Stage III</td>
<td>25</td>
<td>11 (44%) 13 (52%)</td>
</tr>
<tr>
<td>Stage IV</td>
<td>25</td>
<td>19 (76%) 21 (84%)</td>
</tr>
</tbody>
</table>

- a Numbers in parentheses indicate sensitivity in terms of frequency of true positives.
- b Above-normal PSAP > 2.2 µg/L.
- c Above-normal PSAP > 2.0 µg/L.
- d Method of Griffiths (5).

For the positive sera from benign prostate hyperplasia and stages I and II carcinoma, the elevated values were generally close to the cutoff point by both assays. Table 2 shows the distribution of results by test and severity of disease. The concordance was quite low for benign prostate hyperplasia (21%) and stage I (25%), but was substantially better for stages II and IV carcinoma (75%).

**Significance of prostatic mass in benign prostatic hyperplasia.** Increased PSAP concentration in cases of benign prostatic hyperplasia constitutes a false positive if increased PSAP is specific for prostate cancer. However, because benign prostatic hyperplasia is also detectable by rectal examination, we wished to determine the relationship between prostate mass in benign prostatic hyperplasia and serum PSAP concentration. We compared the clinical impression of prostate size (in grams, as estimated by the urologic surgeon at the initial rectal examination) with the PSAP value obtained by ELISA for the same 84 patients' sera (Figure 4). The estimated prostatic mass varied from 15 to 100 g, with estimates for 13 patients (15%) exceeding 50 g. There is an evident trend for PSAP concentration to increase with prostate size for masses exceeding 50 g. Of the 69 patients with PSAP values ≤ 2.0 µg/L, only one had a prostate mass exceeding 50 g. Of the 15 patients with above-normal PSAP values, 11 had estimates of tissue mass greater than 50 g.

**Discussion**

Earlier experiences with an RIA kit (obtained from New England Nuclear) led to the establishment of an upper limit of normal at 7.15 µg/L (5). Because the patients used for the evaluation of normality in the earlier study were essentially similar to those of the present group, we must conclude that the substantial discrepancy between the two studies reflects a change in the composition of the kit in the intervening 24 months. Lee et al. (7), using a solid-phase immunofluorescence assay and subsequently a solid-phase immunosorbent assay, noted an upper limit of normal at 9.89 µg/L. We are not able to explain this markedly different finding.

There may be geographic and racial-related differences in serum PSAP concentrations (9, 10). We urge each laboratory adopting a new procedure to define with an adequate number of patients the reference intervals for the population using that laboratory.

Several authors (3, 5, 9) report that some patients with benign prostatic hyperplasia have a slight-to-moderate increase in serum PSAP. Accordingly, we sought to confirm these findings and to formulate an explanation. Of our patients with histologically proven benign prostatic hyperplasia, 20% had increased PSAP (Figure 4), usually only slightly, and there was a relationship between approximate prostate size and serum PSAP value. We stress that PSAP is not specific for adenocarcinoma of the prostate, although it is abundant in the lysosomes of the prostatic acini in both normal prostatic cells and certain histological types of adenocarcinoma (11). Apparently, an increase in the size and number of the acini in benign prostatic hyperplasia as well as acinar destruction by infection or thrombus will release PSAP into the blood. However, an increased value for serum PSAP in the face of a negative biopsy should not be dismissed, because we cannot with certainty exclude the presence of tumor cell foci.

Examination of our ELISA data relating increases of PSAP concentration to clinical stages suggests that, as tumor mass increases, so does PSAP concentration in those histological types that secrete the enzyme (generally, well- to moderately well-differentiated tumors). The correlation between PSAP values and clinical staging is consistent with other reports (3, 5, 9, 12), and further emphasizes the utility of serum PSAP measurements as a prognostic marker. Clearly, digital rectal examination is still the most reliable method of detecting prostate cancer at stages II, III, and IV, and benign prostatic...
hyperplasia. Increased PSAP values were observed in some stage I and benign prostatic hyperplasia patients. These two populations can be differentiated by rectal digital examination. By definition (2), stage I prostate adenocarcinoma is not detectable by the rectal digital examination, yet it is precisely this group that is most likely to benefit from early detection.

We have carefully considered the small number (25 patients) of stage I prostate adenocarcinoma. The diagnosis was confirmed by surgical procedures after a history that elicited suspicion; of these cases, 24% involved a slight to moderate increase of PSAP. This sensitivity compares well with that in a similar ELISA study by Cooper et al. (9) and recent reports of RIA (3, 5, 12), but poorly with the recently published data by Lee et al. (7), who used both solid-phase immunofluorometry and solid-phase immunoabsorbent assay and found as much as 75% increases of PSAP in stage I disease. We doubt that a small, palpable focus of tumor cells in the prostate gland could in fact cause an increase in PSAP activity to above normal in the 5-L blood pool of an adult. We restate and affirm that position in light of our experience with ELISA. Staging the lesion by digital examination is difficult and does not indicate lymphatic or hematogenous spread of the tumor. That our sera were taken from a library of carefully classified subjects before treatment may explain the differences in results.

From our data we conclude that, owing to the better detection limit and reproducibility of the method at the lower end of the standard curve, the ELISA method is slightly superior to RIA for the diagnosis of early prostate adenocarcinoma not detected by rectal examination. We doubt any other current technique will be more sensitive.

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References