Prostate-Specific Acid Phosphatase: Re-Evaluation of Radioimmunoassay in Diagnosing Prostatic Disease

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The availability of a radioimmunoassay for prostate-specific acid phosphatase has allowed a correlative study between this technique and conventional colorimetric assays in the four clinical stages of prostatic adenocarcinoma. Results of such a study show an increased diagnostic sensitivity of the radioimmunoassay in all stages, but in 14% of Stage IV adenocarcinomas there was no increase in prostatic acid phosphatase above the ranges ordinarily expected for all methods. In cases of benign prostatic hyperplasia, there was an increase associated with in vivo tissue cytology, comparable to Stage II and III adenocarcinoma. The sensitivity of the test in Stage I is still low, but testing for combinations of tumor markers might increase the diagnostic yield. Conversely, a different clinical approach might be to establish baseline values in the "at-risk" patient, followed by regular determinations of prostate-specific acid phosphatase activity. Increases in activity within the normal expected range may be interpreted by the clinician as a herald of disease.

Additional Keyphrases: cancer • RIA and colorimetric assays compared • reference intervals • correlation between clinical staging and enzyme assay results

The association between increased acid phosphatase [orthophosphoric monoester phosphohydrolase (acid optimum); EC 3.1.3.2] activity and prostatic adenocarcinoma with metastatic disease was established in 1938 (1). Four decades of clinical study relating to total acid phosphatase and the prostate-specific fraction (PSAP), involving a variety of laboratory techniques, have not altered the concepts of the Gutmans or provided additional pertinent diagnostic information. At times, the usefulness of acid phosphatase activity as a diagnostic tumor marker has been questioned (2).

Currently accepted methods for total enzyme activity determination, mainly colorimetric, have inherent problems. Despite claims (3), no substrate is specific for the prostatic isoenzyme (4), and tartrate inhibition of the fraction (5) is also semi-specific.

Advent of a method for enzymes that measures not only molecular mass but also a specific tissue isoenzyme opens new horizons for the clinical enzymologist. The technique of radioimmunoassay (RIA) developed initially for peptide hormone analysis, is markedly more sensitive and specific than traditional biochemical methods. I therefore considered that the same technique applied to an enzyme such as PSAP might likewise improve sensitivity and specificity. If so, then clinicians could direct particular attention to Stage I prostatic adenocarcinoma, which is curable (7), but cannot be detected by simple rectal examination; current enzymic techniques also provide little diagnostic information for this stage.

A study of this problem by Foti et al. (8) was reported in 1977; however, their findings have not been confirmed and, in fact, were challenged by Mahan and Doctor (9). In the present study, therefore, I examined a large number of normal individuals, patients with the various clinical stages of prostatic adenocarcinoma, and patients with other neoplastic diseases, by using a sensitive and specific PSAP antibody in a radioimmunoassay technique. The study is thus designed to elucidate the precise role of immunologically measured PSAP in diseases of the prostate gland.

Materials and Methods

Laboratory Methods for Evaluation of PSAP

Thymolphthalein. The acid phosphatase method involving thymolphthalein monophosphate hydrolysis (3) is considered more specific for prostatic acid phosphatase than other substrates. The normal range established by use of this method in the Automatic Clinical Analyzer (DuPont Instruments, Wilmington, DE 19898), as stated by the manufacturer and confirmed at the time of investigation, is 0 to 0.8 U/L.

p-Nitrophenyl phosphate. This assay involves the hydrolysis of p-nitrophenyl phosphate to phosphate and p-nitrophenol (10). The total activity is measured at pH 4.8 and the PSAP fraction, after tartrate inhibition, is determined by the technique of Fishman and Lerner (5). The normal range for total acid phosphatase, established in the laboratory, was 0 to 11 U/L; for the PSAP fraction, 0 to 4 U/L.

RIA. Prostatic acid phosphatase RIA kits were supplied by New England Nuclear, Medical Diagnostics Division, Boston, MA 02118, on a strictly investigational basis. To prepare the kit, acid phosphatase obtained from human seminal fluid was purified by a series of ammonium sulfate precipitations combined with separation on CM-Sepharose, DEAE-Sepharose, and finaly, Sephadex G-25 columns. The purified PSAP was injected into New Zealand white rabbits, and the antibody to PSAP was obtained by standard procedures. PSAP was iodinated by a commercial modification of the Hunter and Greenwood technique, with 125I tracer. Serum to be investigated or standard were incubated with PSAP antibody, and iodinated PSAP for 4 h at room temperature, then free and bound PSAP were separated with a second antibody derived from sheep anti-rabbit-serum. The PSAP standards were supplied lyophilized and were reconstituted with distilled water. I estimated the amount of radioactivity in the precipitate with an analytical gamma counter (Model 1285; Searle and Co., Des Plaines, IL 60018) with a PDS/3 computer (efficiency for 125I, 80%). Studies with erythrocyte and platelet acid phosphatase showed a cross reactivity with the PSAP antibody of less than 1%.

Analytical recovery experiments indicated a percentage recovery of 91 to 110%.

Reproducibility was determined by 20 consecutive analyses of 12 samples. The coefficient of variation ranged between 5 and 9% within-assay, and 7.5 to 10% between assays.

The sensitivity of the method, defined as the concentration equivalent to twice the standard deviation of the zero binding, was 1.0 µg/L.

Figure 1 shows a histogram for 223 men, 20 to 85 years old. The expected normal range, defined by two standard deviations from the mean, was 2.5 to 7.15 µg/L. It was clinically
established that none of the normal men had evidence of prostatic disease. An age breakdown by decades showed no significant difference in immunoassay values among the age groups. Sixty normal women were examined and, although some serum samples showed antigenic activity with the PSAP antisera, in no case did the value exceed 2.5 μg/L.

Studies of Patients

Blood was sampled from each patient at least 24 h after rectal examination (11). Because PSAP is one of the most unstable of the enzymes commonly measured clinically, specific precautions are necessary to ensure stability. For both assays (activity and RIA) blood was collected in an evacuated tube, which was placed in crushed ice. Within 30–60 min the sample was centrifuged at 4 °C and serum was separated from the cells. In the aliquot for the rate-reaction assays, 10 μL of glacial acetic acid was added per milliliter of serum, and the sample was stored at 4 °C. Generally, the activity was assayed within 4 h of blood sampling. For radioimmunoassay, the serum was stored at −70 °C without acetic acid. Assays were performed in a batch about twice weekly, the average storage time being 72 h. Stability studies over a 24-h period indicated antigenic activity was retained without loss at 4 °C in non-acid-treated serum. Fresh serum stored at −70 °C retained antigenic activity for at least three months, as has been described for the acid-stabilized serum used in colorimetric assays (12).

Staging of the Patients

All patients with benign prostatic hyperplasia and adenocarcinoma were examined by histological section of tissues after surgery. The clinical staging of those cases with adenocarcinoma were confirmed by initial rectal examination, prostatic surgery, bone-marrow examination, pelvic lymphadenectomy, and bone scan.

Results

Table 1 shows comparative results between the various assay methods for normal men and women, patients with benign prostatic hyperplasia, patients after total prostatectomy, and patients with various non-prostatic neoplastic diseases.

The 213 cases of histologically confirmed benign prostatic hyperplasia, 19 (9%) showed increased PSAP, with values as high as 30 μg/L by RIA. Histological examination showed, in 17 cases, evidence of macro- or micro-infarction with areas of prostatic cytolysis.

In the 10 patients who had undergone total prostatectomy, each more than six months before serum examination, seven showed detectable antigenic PSAP, ranging between 0.5 to 2.5 μg/L. None, however, had concentrations exceeding 2.5 μg/L.

In 109 patients with both solitary and disseminated non-prostatic neoplastic diseases, 10 patients (9%) had increased PSAP activity, as determined by RIA, in the range of 7.5 to 11.0 μg/L. These cases included four oat-cell carcinomas of the lung, one renal clear-cell carcinoma, one adenocarcinoma of the pancreas, one adenocarcinoma of the female breast, two intestinal carcinoids, and one Hodgkin’s lymphoma. In the male patients, there was no clinical evidence of prostatic disease.

Table 2 shows comparative results in patients with histologically proven adenocarcinoma of the prostate. These cases have been placed in the four clinically staged groups. In addition, Stage I disease has been histologically subdivided into stage Ia, representing microfoci of tumor cells, and Stage Ib, a more diffuse extension of the tumor pattern but confined within the prostatic capsule.

In Stage IV, there were 10 cases in which all tests for PSAP were negative, despite widespread extension of the carcinoma.

Sensitivity and specificity were calculated from the accumulated data in the manner described by Galen and Gambino (13). The sensitivity data for each clinical stage of prostatic

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Table 1. Normal Ranges and Number of Patients with PSAP Exceeding Established “Normal Range” as Determined by Three Methods

<table>
<thead>
<tr>
<th></th>
<th>No. cases</th>
<th>PSAP, μg/L, by RIA</th>
<th>Thymolphthalein</th>
<th>Total</th>
<th>Prostatic fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ranges:</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Men (20–85 years old)</td>
<td>223</td>
<td>2.5–7.15</td>
<td>0–0.8</td>
<td>4–11</td>
<td>0–4</td>
</tr>
<tr>
<td>Women (20–85 years old)</td>
<td>60</td>
<td>0–2.5</td>
<td>ND</td>
<td>0–3</td>
<td>ND</td>
</tr>
<tr>
<td>Benign prostatic hyperplasia</td>
<td>213</td>
<td>19 (9%)</td>
<td>11 (5%)</td>
<td>0</td>
<td>9 (4%)</td>
</tr>
<tr>
<td>Total prostatectomy</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Other cancers</td>
<td>109</td>
<td>10 (9%)</td>
<td>3 (3%)</td>
<td>0</td>
<td>2 (2%)</td>
</tr>
</tbody>
</table>

Incidence of positive results in patients:

adenocarcinoma are outlined in Table 2. Specificity was 94% for the RIA and 92% for the thymolphthalein assay in the four clinical stages of prostatic adenocarcinoma.

In one case of Stage IV disease, the radioimmunoassay value was within the normal range, but there was increased PSAP activity, as determined with thymolphthalein and para-nitrophenol. Improper sample handling may have been responsible for this false negative by RIA.

In two cases within the clinically normal group, RIA gave above-normal results exceeding 100 μg/L, but the colorimetric methods indicated normal activity. In both cases, the subjects were hyperlipidemic, with cholesterol >5.0 g/L and triglycerides >8.0 g/L. This would suggest that hyperlipidemia affects the technical performance of the RIA test.

Table 3 compares our diagnostic results with those of three previous authors. In Stages I, II, and III, there appears to be a wide variation in diagnostic results, especially compared with the report of Foti et al. (8), who used a similar RIA technique. Our data agree better with those of Chu (14), who used a counter-current immunoelctrophoresis method, and Mahan (9), who used RIA.

With a statistical analysis of the data I sought answers to two questions:

(a) Is the RIA method significantly better than the colorimetric activity assays in detecting cancer at the various stages? The p-values from McNemar's test (15) for correlated proportions (Table 4) is compelling evidence for the combined data and strong for each individual stage except Stage I, which is, unfortunately, of particular interest. However, in view of the statistical significance for Stages II and IV, the lack of statistical significance for the Stage Ia results appears to be due to insufficient sample size.

(b) Is the RIA method effective for detecting Stage I cancer? It must be determined whether the true-positive rates for Stage I are (statistically) significantly higher than the false-positive rate of 12/223 I found for normal subjects. Using the standard test for the difference of independent proportions, I obtained the following p-values:

<table>
<thead>
<tr>
<th>Stage</th>
<th>True positive</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Ia</td>
<td>1/20</td>
<td>0.53</td>
</tr>
<tr>
<td>Ib</td>
<td>6/38</td>
<td>0.01</td>
</tr>
<tr>
<td>I (combined)</td>
<td>7/58</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Thus, there is strong evidence that the RIA is effective for detecting Stage Ib adenocarcinoma; in this small sample series, however, there is no statistical indication that Stage Ia can be detected by RIA.

Discussion

In the decision to introduce a new laboratory test or replace an older one, several criteria must be evaluated.

Does the new test offer advantages of specificity and sensitivity? The present data, in which an RIA-specific antibody technique is compared with common colorimetric enzymic activity assays, show a definite improvement in sensitivity but a less significant change in specificity.

Does the new test offer the advantage of reliability in repetitive testing? Experience over a six-month period suggests that there are few methodological false-positives or false-negatives with the test. A high concentration of lipids causes false-positives, and improper sample handling may account for false negatives.

Finally, does the test offer better diagnostic accuracy than other procedures used in investigating the same disease process—i.e., fewer false pathophysiological positives? A few (9%) by this RIA of those cases histologically determined to have benign prostatic hyperplasia showed enzyme activity that was above the normal range, which by tradition suggests neoplasia. Most of these cases had either macro or micro areas of infarction associated with areas of cellular necrosis, a finding that had been previously reported by Howard and Fraley (16). In 14 of the 19 cases, an indwelling urethral catheter had been in place for at least 72 h before PSAP activity was determined. Nonetheless, the lingering doubt of a missed focus of tumor cells remains and can be evaluated only by continued clinical and laboratory observations.

A broad variety of other neoplastic diseases appear to re-
lease an acid phosphatase that is antigenically similar to PSAP. The concentration of this enzyme does not appear to be high (<11 μg/L) despite widespread disease in some cases. This observation must remain a curiosity at present, with minimal diagnostic value for those particular tumors. It does, however, remind us that isoenzymes may not be totally tissue-specific.

The consistent finding of low antigenic activity both in post-prostatectomy patients and in women also suggests that the present antibody is not completely specific for PSAP.

On critically examining the data for adenocarcinoma of the prostate, certain positive conclusions can be drawn. In Stages II, III, and IV, despite an improved enzymatic sensitivity corresponding with extension of the disease—i.e., increases in tumor burden—accuracy of the enzyme evaluation still cannot compare with that of a properly performed rectal examination, followed by needle biopsy or other form of biopsy directed toward the suspicious palpable areas. I re-emphasize that in a significant proportion of Stage IV prostatic adenocarcinomas (14%), even with extensive metastatic disease, there is no apparent increase in serum PSAP. On histological section of these tumors, 90% were of the anaplastic variety, Gleason Type 5, with the remaining varying between Gleason Type 3 and 4. My observations of sera, coupled with histological confirmation, agree with the elegant histochemical studies of Gyorkey (17) and imply that in a significant number of cases, the tumor does not release a detectable amount of enzyme.

In Stage I disease, which is considered curable (but not diagnosable by rectal examination), even a 12% positive diagnostic yield is a moderate advantage, compared with the 5% yield of colorimetric techniques. The data I present do not compare well with those of Foti et al. (8), who claimed 33% of Stage I patients showed an increase in the activity of PSAP by RIA. Several reasons may account for the difference in results. Clinically, the rigid staging procedure I applied is critical to the conclusions. Methodologically, although every precaution was taken to ensure enzyme stability, it is conceivable that there are differences in specificity and sensitivity between the two antibodies used, because a more extensive purification procedure basis was used for the New England Nuclear antibody than for the Foti one.

The results I present correspond closely to those of Mahan and Doctor (9) and to those of Chu (14). Only considerable further practical experience will answer the question.

Whichever study one accepts, one cannot be euphoric over the positive diagnostic yield of 12%. The realistic question that must now be asked, in view of the accumulated data by a clearly improved methodological approach is: are we examining the right biochemical parameters?

Is it possible for any biochemical technique, even with superb specificity and sensitivity, to detect the enzyme output of a small focus of tumor cells in the prostate (or any other organ) after dilution in a 5-L blood pool, and at pH foreign to that of its natural milieu? One must accept the limitations, both physiological and methodological.

There is slight evidence that an increase in the number of tumor markers examined in combination with PSAP, such as ribonuclease (14), creatine kinase isoenzyme I (BB) (18), and lactate dehydrogenase isoenzymes 4 and 5 (19), may increase diagnostic sensitivity. Or there may be a different clinical approach. All the data in this study, as in past studies, have taken single-point analysis of enzyme activity, and deviation above the upper limit of normal in this analysis, as the index of abnormality. For individuals "at risk" this may be a serious error in judgment: a subtle increase within the normal range may be more significant. For example, an individual monitored over a period of apparent good health will show a consistent baseline PSAP. If samples taken under identical circumstances (20) to the baseline samples show increases that persist over time, yet remain within the normal range, such changes may have diagnostic significance.

Perhaps we should consider the estimation of PSAP activity the male counterpart of the Papanicolaou smear, and apply a similar routine application in the defined "at-risk" population—i.e., American blacks from age 45, American whites at 50, and so on.

I particularly appreciate the efforts of Mr. Thomas Leech and Dr. Karen Whitlow for continued expert technical assistance, and of Dr. Henry Tan, Director of Laboratories, Palomar Hospital, Escondido, CA, for willing participation in the patient population study.

References