Acid Phosphatase: Clinical Utility of the First Tumor Marker

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Acid phosphatase was the first tumor marker to be measured in blood. Although it is used as a marker of prostatic carcinoma, serum levels are also elevated in many diseases of prostate, bone, and hematologic tissue. While serum acid phosphatase concentration is elevated in patients with prostatic carcinoma or infarction, it may also be increased merely with benign hypertrophy and after prostate massage. Recent studies demonstrate that levels are not significantly increased after routine rectal examination. Although of limited use as a screening test, the assay may be useful in staging, prognostication during hormonal therapy, and in making clinical decisions. Rectal examination remains the best screening test for carcinoma of the prostate.

Carcinoma of the prostate is the second most common cause of cancer death among American men, with 42,000 new cases and 17,000 deaths per year (1,2). It has been estimated that 30% of new cases are potentially curable (3,4). Digital examination of the prostate has been the standard screening test, but it has been reported that as many as 80% of patients have advanced disease on initial presentation (5). Thus, prostatic carcinoma, although often detected early as a nodule in the gland, continues to be a difficult problem for the primary care physician. Given the difficulties of anatomic tumor detection, attention has turned to the use of tumor markers in this condition, and the measurement of serum acid phosphatase (SAP) has been widely used. Although assay methodology has advanced in recent years, several problems remain in using SAP as a marker both for screening and corroboration of diagnosis. This is well illustrated by the following case report.

Case Report

A 70-year-old white man presented with fatigue, a 40-pound weight loss, bone pain, and polyuria of 6-12 months' duration. His past medical history included diabetes mellitus type II controlled with diet and chlorpropamide 500 mg daily, coal miner's pneumoconiosis, and hypothyroidism, for which he was receiving desiccated thyroid. In 1974, he had undergone cholecystectomy, vagotomy, and pyloroplasty for cholelithiasis and a duodenal ulcer.

Physical examination disclosed pallor and tenderness of the sternum and left ribs. The prostate was enlarged and the right lobe was indurated. There was mild distal sensory neuropathy.

Hemoglobin was 9.5 g/dl and the leukocyte count was 8400/cu mm with 36% polymorphonuclear forms, 10% bands, 30% lymphocytes, 16% monocytes, 1% eosinophils, 4% atypical lymphocytes, 1% metamyelocytes, 1% myelocytes, and 1% promyelocytes. The platelet count was 70,000/mm³. Urinalysis disclosed 1+ protein and 2+ glucose reactions, 2 red blood cells, and 4 leukocytes per high powered field. Electrolyte concentrations were normal, fasting blood glucose was 300 mg/dl, calcium was 9.2 mg/dl with an albumin of 4.0 g/dl, and phosphate was 4.0 mg/dl. The alkaline phosphatase was 185 u/L (normal: 35-115), serum aspartate aminotransferase (SGOT) was 10 u/L (normal: 9-33), and lactic dehydrogenase was 1106 u/L (normal: 106-290). The acid phosphatase assayed by thymolphthalein monophosphate substrate was 21.8 u/L (normal: 0-0.7).

Although chest and left rib roentgenograms were nor-
Clinical Use of Acid Phosphatase

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A 99mTc methylene diphosphonate bone scan revealed increased activity in the left lower ribs. A 99mTc sulfur colloid liver/spleen scan demonstrated non-visualization of the left lobe of the liver and a defect on the medial aspect of the spleen. Based on clinical diagnosis of carcinoma of the prostate with bone metastases, transurethral resection of the prostate was performed. The tissue diagnosis was benign prostatic hypertrophy. Subsequent bone marrow biopsy revealed malignant histiocytosis.

Discussion

Acid phosphatase activity (EC 3.1.3.2) refers to the ability of a group of enzymes to hydrolyze phosphate esters in an acid environment. In 1924, the activity was first discovered in erythrocytes, and in 1935, intermittent surges of acid phosphatase activity originating from the prostate was demonstrated in the urine of men. From 1936 to 1942, Gutman (6) studied serum acid phosphatase in human disease and found high levels in patients with prostatic carcinoma, particularly those with bone metastases. In this way, the measurement of serum acid phosphatase as the first tumor marker was established. In 1941, Huggins (7) published his classic paper demonstrating that the prostatic epithelium is under hormonal control and that in patients with prostatic cancer, castration and estrogen administration lower SAP, while androgens raise the level. These observations form the basis for modern hormonal therapy.

While much is known about the activity of these enzymes in vitro, their in vivo function is unknown, because all in vitro studies have used artificial substrates in a hostile acid environment. Acid phosphatase is present in all tissues and body fluids. Gram for gram, the normal prostate contains a thousand times more of the enzyme than any other tissue. In the prostate, the enzyme is found in the epithelium and lumen where it is primarily a secretory product. In the reticuloendothelial system, acid phosphatase is a lysosomal enzyme that accounts for the activity in the liver and spleen. Other cells containing significant quantities include osteoclasts, granulocytes, and platelets (8).

Polyacrylamide gel electrophoresis has demonstrated the heterogeneity of the acid phosphatases from various tissues (Table 1). By means of isoenzyme analysis, it can be demonstrated that in prostatic carcinoma metastatic to bone the elevated SAP is due to prostatic acid phosphatase, while in other bone disease, including metastases from nonprostatic carcinoma, the SAP originates from osteoclasts. Sensitive techniques demonstrate low SAP activity in normal persons. Plasma contains fraction 5, while serum contains fractions 3 and 5. Therefore, osteoclasts and platelets rather than prostate cells account for normal SAP activity. This cumbersome technique is not readily available for clinical use.

Clinical enzymatic assays employ a phosphate ester substrate which when hydrolyzed by phosphatase in the patient's serum yields a colorimetrically measurable product. Substrates used include phenylphosphate (King Armstrong units), B-glycerol phosphate (Bodansky units), nitrophenyl phosphate, and more recently, thymolphthalein phosphate (international units). The last is the most popular substrate used at present and probably the most specific for prostatic acid phosphatase isoenzymes (9).

Since the development of antibodies to prostatic acid phosphatase in 1972, several immunoassays have been developed. These include competitive binding radioimmunoassay (RIA), enzymoimmunoassays, and immunohistologic methods (10).

The instability of SAP by enzyme assay has been a major drawback to its clinical use. When separated from the clot at room temperature, activity is attenuated in one to

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<th>Table I: Polyacrylamide Gel Electrophoresis</th>
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<tr>
<td>Isoenzyme</td>
</tr>
<tr>
<td>0  Gaucher's cells</td>
</tr>
<tr>
<td>1  tissue bound</td>
</tr>
<tr>
<td>2  prostate, leukocytes</td>
</tr>
<tr>
<td>3  platelets, other tissues</td>
</tr>
<tr>
<td>4  prostate, other tissues</td>
</tr>
<tr>
<td>5  osteoclasts, histiocytes</td>
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modified from Yam (8)
two hours but is well preserved when buffered to a pH of 6.2 to 6.4. Because SAP concentration is altered not only by enzyme inactivation but also by the contribution of platelet enzyme, the ideal specimen is a fresh plasma sample buffered to a pH of 6.4 (8). Radioimmunoassay is not affected by enzyme instability.

**Acid phosphatase in nonprostatic disease**

Table II lists the major diseases that are accompanied by elevations in SAP. In bone disease other than metastatic prostatic carcinoma, acid phosphatase is of electrophoretic group 5 and reflects osteoclastic activity. Alkaline phosphatase reflects osteoblastic activity. Both enzymes may be complementary for the early detection of bone metastases in breast cancer patients (11). Miscellaneous conditions for which scanty data exist include multiple myeloma, osteoporosis, osteogenesis imperfecta, and thyroid and renal osteopathy (12).

The frequency of SAP elevation in Gaucher’s disease depends upon the substrate used; it approaches 100% with phenylphosphate but only 20% with glycerol phosphate. Increased SAP has not been reported in Tay-Sachs or Letterer-Siwe disease.

The elevations in SAP induced by platelet lysis during clotting are usually small (approximately 0.15 IU/ml), unless thrombocytosis is present (13). Thrombocytosis does account for the elevated SAP in thrombocytopenia, polycythemia vera, and chronic granulocytic leukemia. Increased concentrations found in patients with myeloid metaplasia and acute lymphoblastic leukemia, the lymphoproliferative disorders fail to produce elevations in SAP (14). Platelet lysis may produce mild elevations in thromboembolic disorders. There are anecdotal reports of SAP elevations in liver disease and in the multiple endocrine neoplasia syndrome (15). A familial deficiency of lysosomal acid phosphatase has been reported that presents as failure to thrive in infancy (16).

**Acid phosphatase in prostatic disease**

While SAP activity in nonprostatic disease is fascinating from a biological viewpoint and essential to understand when levels in an individual patient are to be interpreted, the ultimate use of the assay is as a marker of prostatic carcinoma; indeed, assay methods have been directed toward this end. There remain, however, several areas of confusion.

In 1949, after Hock and Tessler (17) had observed discrepancies in the SAP of a patient who had undergone prostatic massage and catheterization for urinary obstruction, they studied the effect of prostatic massage on the SAP of 20 patients with prostatic disorders. Although a rise in SAP occurred in 17 patients, only four achieved levels compatible with a diagnosis of prostate disease.
carcinoma. However, the authors recommended that the determination of SAP be delayed 24 hours after prostatic massage. While this effect of massage has been corroborated by some (18,19) and appears to be accepted by many urologists (20), some recent authors (21) have failed to find SAP elevations. These early studies employed assay methods now known to have poor reproducibility, especially at low normal levels (22). Nevertheless, the concept that routine rectal examination with palpation of the normal prostate may cause elevations in SAP is widespread (23). Although many recent studies refute this belief (20-25), it persists tenaciously as an aphorism taught to medical students.

Some early authors concluded that a rise in SAP after rectal examination indicated prostatic carcinoma (26,27), but recent studies have also failed to corroborate this concept (20,24). One source of difficulty in establishing a relationship between SAP levels and prostatic carcinoma is the wide random fluctuations which occur normally (20,28). In addition, after transurethral resection of the prostate performed on patients either with benign hypertrophy or with carcinoma, SAP elevations of 150-fold normal occur in the former, while no elevation is observed in those with malignancy. The difference could be due to more limited resection of the carcinomatous gland, but this variable has been acceptably controlled. Very likely, the normally differentiated benign gland contains more phosphatase than does malignant tissue (29).

It has been fairly well documented that infarction of the prostate can cause SAP to rise four to seven times normal. For example, 8.3% of patients with benign prostate hypertrophy were found to have elevated SAP; in all cases, infarction was documented histologically (30). Silber (31) reported elevated SAP in 30% of those with infarction. Many such patients present with acute outlet obstruction from the edema of infarction after a long history of prostatitis. It is likely that the hypertrophied gland outgrows its blood supply and releases acid phosphatase after infarction.

**Correlation with disease activity**

The studies of Huggins (7) in 1941 established the rationale for hormonal therapy of prostatic carcinoma and proposed a use for SAP as a marker of disease activity. However, only 75-85% of patients with an advanced stage of the disease have elevated SAP. The possible explanations for this variation, such as lack of secretion with differentiation, low rates of secretion, or some barrier to secretion enzyme, are all unproved. Moreover, SAP levels fluctuate randomly by 44-97%, making interpretation very difficult (28). Ishibe (31) observed that the SAP in patients with stage B, C, and D carcinoma did not correlate with five-year survival during stilboestrol treatment. The report has been corroborated but has not been correlated with histological grade, primarily because of technical difficulties (32). However, 14% of patients with stage D disease reported by Griffiths (11) had normal SAP, and 90% were classified histologically as anaplastic. Furthermore, during anti-adrogenic therapy, patients who exhibit a rise in SAP have a poor prognosis, while those in whom SAP falls have an improved prognosis (31,32). In those who relapse after hormonal therapy, a fall in SAP may correlate with survival, a 50% reduction in tumor mass, and relief of pain after chemotherapy (30).

**Acid phosphatase as a screening test**

Until 1977, it was believed that elevated SAP by enzyme assay signified the presence of extracapsular, hence incurable, disease. That year, in a pilot study employing radioimmunoassay, Foti (33) concluded that over one-half of all intracapsular disease could be detected. Therefore, SAP by radioimmunoassay was recommended as a screening procedure for intracapsular disease, even that undetectable by rectal examination, which was referred to as the "male PAP test" (34).

Despite the difficulties of the reported studies, there are several points of agreement. Nevertheless, Foti's optimistic report on the sensitivity for the diagnosis of intracapsular disease has not been confirmed. Although the sensitivity of RIA is better than that of enzyme assays, it cannot signal the presence of intracapsular disease. Moreover, the specificity of RIA is only marginally better than that of the enzyme assay.

The predictive value of SAP can be calculated using data for the incidence of prostate cancer, with the sensitivity and the specificity of RIA. The prevalence of the disease in the U.S. adult white male population was 35 per 100,000 in 1964. Radioimmunoassay sensitivity of 70% and specificity of 94%, determined by Foti (33) for all stages, can be used. Thus, the predictive value of this test will be 0.41% for the male population; that is, one of every 244 subjects with elevated SAP will have prostatic carcinoma. Even if the specificity of the test reached 100%, the cost of detecting one case of prostate carcinoma by screening blood tests would be $36,000. Clearly, the test has little utility for screening an unselected population. If used to screen only men 50 to 85 years of age, i.e., those at highest risk, the predictive value remains

*See the Appendix for a discussion of the sensitivity and specificity of SAP by RIA and by enzyme assay.
only 7%. To obtain a predictive value of 50%, as accurate as the flip of a coin, even in this high risk population, a sensitivity of 98% and a specificity of 99.5% would be required (35). Statistical considerations alone demonstrate the futility of SAP screening tests.

On the other hand, if the disease prevalence is increased by further selection, the predictive value of SAP assay is improved considerably. The probability of malignancy in a given prostatic nodule is 50%. In this population, the predictive value of a positive test is 93% and of a negative test is 84%. This information is particularly useful in evaluating a patient with a nodule when needle biopsy demonstrates benign hypertrophy. If SAP is above normal, the probability of carcinoma is 93%, and the patient should have a second biopsy. If the SAP is normal, the probability of benign disease is 84%, and multiple biopsies are not indicated (36).

Guinan (37) compared prospectively ten screening tests for prostatic carcinoma. The population consisted of 300 men between the ages of 50 and 90 who were examined for symptoms of urinary obstruction. Final determined prevalence of carcinoma was 0.23%. Staging was not reported. On the basis of rectal examination, 85% of patients were classified correctly. SAP by enzyme assay classified 84% correctly, while SAP by RIA was only 70% accurate. The reasons for the poor sensitivity and specificity of RIA in this study are unclear. Rectal examination in this study was performed by a urologist, and patients were selected because they had symptoms of disease. The qualifications of the examiner may explain the superior accuracy of the rectal exam, but the state of the patients would be expected to enhance the efficiency of all tests. While the problem of asymptomatic patients is not addressed in this study, the rectal examination is clearly the simplest, least costly, most reliable screening test available.

Lindholm (38) questions the very concept of detecting intracapsular disease. He argues that such a small tumor focus should not be expected to raise SAP above threshold when the neoplasm produces less enzyme on a gram-for-gram basis than the normal or hypertrophic gland. Moreover, specificity of the test is not perfect; the secreted enzyme is diluted in five liters of blood and then measured in a nonphysiologic environment. While SAP may be of some utility in staging, prognosticating during therapy, and clinical decision making, it is irrelevant to diagnosis.

Appendix

The utility of a test for screening purposes is a function of its predictive value, which is dependent upon its sensitivity and specificity as well as the prevalence of the disease in the population to be screened (38).

Table III defines the relevant statistics. In the evaluation of a diagnostic test, the initial step is to determine how often the test is abnormal in a population with well-defined disease; the sensitivity and specificity so determined are useful in comparing tests to each other. In practice, however, the question is not whether the person with disease has an abnormal test but whether the person with the abnormal test has disease. In a population with a low prevalence of disease, the positive predictive value depends more on prevalence and specificity than on sensitivity.

The major studies of the sensitivity and specificity of SAP both by RIA and by enzyme assay in the diagnosis of prostatic carcinoma are chiefly impressive in the wide divergence of the data. Several recent reviewers have discussed immune methodology (10,40,41,42,35). While most employ double antibody radioimmunoassay, Chu (43) used counterimmunoelectrophoresis, an assay known to be less sensitive (10). To improve specificity, most assays use human prostatic fluid as a source of antigen. Chu (43) used malignant prostatic tissue as a source of antigen, although there is no evidence for an acid phosphatase specific for malignancy. The concept of a unique prostate-specific acid phosphatase is unproven, for low levels of SAP by

<table>
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<td>Calculation of the Predictive Value of a Single Laboratory Test</td>
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| Se | probability that a person with the disease has a positive test |
| Sp | probability that a person without the disease has a negative test |
| PVpos | probability that a person with a positive test has the disease |
| P | prevalence of the disease in the population |
| TP | true positive |
| FP | false positive |
| TN | true negative |
| FN | false negative |

74
Sensitivity and specificity are interdependent and their calculation is based on data including the presumed upper limit of normal. Published studies are frequently not comparable because the selected "normal" populations differ. Foti (33) set the upper limit of normal above that of the entire normal population, which selected "normal" populations differed. Griffiths (34) chose as a threshold value the upper limit of normal based on data including the presumed normal population.

In addition to the use of differing control populations, failure to include staging criteria contributes to the varying reliability reported for SAP assay as a diagnostic test for prostate carcinoma. Undertaging, which is common when clinically staged patients are subsequently pathologically staged, will increase the apparent sensitivity for early disease (41). Of Lindholm's 98 patients (38), 43 were staged pathologically. While he found sensitivities of 22% and 29% in stages A and B, respectively, none of the seven pathologically staged patients in his group had a positive test, and none of those with a positive test were pathologically staged. Bruce (44) staged the group with intra-prostatic disease by bimanual exam. While Foti in his original paper made no comment on staging details, he later confirmed that he staged laparotomy for stage B disease (45).

Aside from simple lack of specificity, the only other proposed etiology of false positivity is hyperlipoidemia, which Griffiths (34) found in two of his normal control group. While SAP was markedly elevated by RIA, the enzyme assay was normal.

**References**


38. Lindholm GR, Stirton MS, Liedtke RJ, Batjer JD. Prostatic acid phosphatase by radioimmunoassay. JAMA 1980;244:2071-3.


