Extremely High Values of Prostate-Specific Antigen in Patients with Adenocarcinoma of the Prostate; Demonstration of the "Hook Effect"

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We reviewed 721 consecutive samples submitted for measurement of prostate-specific antigen (PSA) over five months. We identified three patients with extremely high PSA concentrations: 650, 1840, and 3280 μg/L (their acid phosphatase activities were 3.2, 1337, and 2.8 U/L, respectively), and present case reports for the latter two. Serial dilutions of samples obtained from the patient with the highest PSA concentration indicated that the one-step Tandem®-PSA assay gave falsely low values for high concentrations of PSA, an observation consistent with the phenomenon of the "hook effect." This effect was not observed when the sample was reanalyzed for PSA by a two-step procedure.

Additional Keyphrases: prostate adenocarcinoma • acid phosphatase • analytical error • radioimmunoassay

Adenocarcinoma of the prostate is the second most common cancer in males in the United States (1). In the advanced stages of this disease, the patients often show increased concentrations of prostatic acid phosphatase (EC 3.1.3.2) and prostate-specific antigen (PSA) (2–6). These biochemical markers, particularly PSA, have been proposed as an adjunct to monitor tumor recurrence in patients with prostatic cancer (2, 3). Despite the increasing use of PSA measurements, high values of PSA are not frequently reported in the literature. Since the introduction of the PSA assay five months ago in our clinical laboratory, we have identified three patients with extremely high PSA concentrations (>600 μg/L). Herein we describe two cases of prostatic cancer in patients with high PSA values.

Case 1

A 60-year-old man presented at a primary care center with gross hematuria without complaint of obstructive uropathy. Physical examination revealed an enlarged, mobile, benign-feeling prostate without any evidence of pelvic mass or fixation. Computerized tomographic scan revealed an enlarged prostate and the 99mTc bone scan demonstrated multiple metastases. Total acid phosphatase activity in serum was 1089 U/L (normal range, 0–0.7 U/L), but needle biopsies of the left and the right lobes of the prostate showed no malignancy. However, because of continued suspicion of prostatic malignancy, the patient was transferred to Barnes Hospital for further evaluation. Laboratory values before rectal examination confirmed the high activity of total acid phosphatase (1337 U/L) and showed a high concentration (1580 μg/L) of PSA (reference range, 0–4 μg/L). The only other abnormal laboratory results were increased activities of alkaline phosphatase, 282 U/L (reference range, 45–115 U/L), and lactate dehydrogenase, 334 U/L (reference range, 100–250 U/L). The needle biopsy repeated at our hospital demonstrated moderately differentiated prostatic adenocarcinoma. The patient then underwent bilateral orchietomy. Serum values of acid phosphatase and PSA had decreased markedly three weeks after the procedure (Table 1).

Case 2

A 67-year-old man came to Barnes Hospital three years ago with a complaint of right knee pain. He was subsequently diagnosed as having poorly differentiated adenocarcinoma of the prostate and was treated with primary radiation therapy. On this admission, a 99mTc bone scan showed multiple metastases. Acid phosphatase activity in the serum was 14.6 U/L, with a PSA concentration >1020 μg/L (Sample A, Figure 1a). The exact value of PSA could not be determined because the serum sample was not available for further dilution. The patient then underwent bilateral orchietomy. Six months later, his values for acid phosphatase and PSA were 2.8 U/L and 3280 μg/L, respectively (Sample B, Figure 1a).

Materials and Methods

We measured acid phosphatase as described by Roy et al. (7) with thymolphthalein monophosphophate as the substrate, in an acu IV analyzer (DuPont Diagnostic Systems, Wilmington, DE 19898). Total acid phosphatase was also measured by SmithKline BioScience Laboratories (St. Louis, MO 63132) with p-nitrophenyl phosphate as the substrate (kit no. 104; Sigma Chemical Co., St. Louis, MO 63178); they measured prostatic acid phosphatase by radioimmunoassay, following the procedure of Foti et al. (8).

We measured PSA by an immunoradiometric (sandwich) assay (Tandem®-PSA; Hybritech Inc., La Jolla, CA) involving two monoclonal antibodies that recognize different epitopes on the PSA molecule. We performed this assay in one step as recommended by the manufacturer or modified it to two steps. In the modified assay, we incubated the antibody-coated bead for 2 h at room temperature with 50 μL of samples or standards and 200 μL of phosphate-buffered saline containing bovine serum albumin (PBS-BSA; 50 mmol NaPO4, 0.15 mol NaCl, and 10 g bovine serum albumin per liter of de-ionized water, pH 7.4). After washing the beads twice with 1 mL of the kit's washing solution, we incubated them with 200 μL of 125I-labeled anti-PSA antibody (276 985 counts/min) and 50 μL of PBS-BSA for 90 min at room temperature. We then washed the beads again, as described above, and measured with a gamma-counter the radioactivity bound to the beads.

Results by the two-step procedure varied linearly with PSA concentration up to 100 μg/L. The PSA values for five patients' samples by this method (y) (range 0.2–66 μg/L) compared well with those obtained by the one-step assay (x): y = 0.98x + 0.3 μg/L (r = 0.999).

References

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Table 1. PSA and Acid Phosphatase Values in Case 1

<table>
<thead>
<tr>
<th>Date</th>
<th>PSA, µg/L</th>
<th>Acid phosphatase</th>
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<tr>
<td></td>
<td>With TMP as substrate, U/L</td>
<td>With PNP as substrate, U/L</td>
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<td>Normal reference interval</td>
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<tr>
<td>10/23/87</td>
<td>116</td>
<td>—</td>
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*Performed at SmithKline BioScience Laboratories. High acid phosphatase activity measured with thymolphthalein monophosphate (TMP) substrate was confirmed with an alternative substrate (p-nitrophenyl phosphate, PNP) and by a radioimmunoassay.

Results and Discussion

Although histological examination of the prostate is required to establish the diagnosis of adenocarcinoma, needle biopsy can miss an area of malignancy (9), as occurred in Case 1. Consequently, serum values of the markers for prostatic cancer (e.g., acid phosphatase and PSA) can help support the diagnosis (4–6). Serial measurement of PSA (10), the most specific marker for prostatic carcinoma (2, 3, 5, 6), can be very useful in monitoring the progression of the cancer and the success of therapy. To our knowledge, the highest published value for PSA is 600 µg/L (5). However, in a five-month period, involving 721 measurements, we found 28 cases with PSA >100 µg/L and three cases with PSA >800 µg/L: 650, 1840, and 3280 µg/L. Given this high frequency of samples with high PSA, we investigated whether such high concentrations could pose any analytical problem.

We found that measuring high concentrations of PSA by a one-step immunoradiometric assay could potentially present an analytical problem. As shown in Figure 1a, serum samples from the patient described in Case 2 gave an apparently lower PSA value when undiluted (Sample A, 106 µg/L; Sample B, 184 µg/L) than when diluted 5- or 40-fold (Sample A, >1020 µg/L; Sample B, 3280 µg/L). Because the PSA value of the undiluted Sample A was very close to the upper limit of linearity of the assay (100 µg/L), we considered that a major underestimation could have occurred. The measured PSA concentration in samples that were diluted as much as fivefold increased with increasing dilution (decreasing concentration of PSA). These results are consistent with the phenomenon of the "hook effect," in which high concentrations of an analyte give an artifactualy lower value when measured by sandwich assays. This effect has been attributed to heterogeneity in the affinity of the immobilized antibody, inadequate washing, or insufficient concentration of labeled antibody (11–14).

To confirm that the "hook effect" was involved in our observations we modified the Tandem-PSA assay to a two-step procedure and assayed a serum sample (PSA concentration 6600 µg/L) from a new patient with prostatic cancer. We assayed several dilutions of this sample with either the one-step or the two-step assay. As shown in Figure 1b, the measured PSA concentration obtained in the undiluted duplicate samples by one-step assay were 78 and 121 µg/L (average 99 µg/L). One of the two PSA values and the average PSA concentration were within the linear range of the assay (100 µg/L). On the other hand, the duplicate PSA values obtained with undiluted sample by the two-step assay were 207 and 220 µg/L (average 214 µg/L). These results suggest that the one-step Tandem-PSA assay can give artifactualy lower values in samples with extremely high concentrations of PSA, and that this artifact can be prevented by modifying the Tandem-PSA assay to a two-step procedure.

Alternatively, samples should be analyzed before and after dilution to be certain that erroneously low values are not reported. We currently use the latter procedure in our hospital. Our experience suggests that high PSA values are not rare, and that users of the Tandem-PSA assay should be aware of this potential analytical problem.
References