Evaluation of a Monoclonal Antibody-based Immunoradiometric Assay for Prostatic Acid Phosphatase

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This report evaluates a new immunoradiometric assay for prostatic acid phosphatase in serum, based on a dual monoclonal antibody reaction system (Hybritech-TANDEM). A solid-phase antibody binds the acid phosphatase molecule and a second monoclonal antibody to a different antigenic site serves as the $^{125}$I-radiolabel. The method was tested on 67 patients with various stages of prostatic carcinoma and 134 patients without the disease. It also was compared with a conventional polyclonal radioimmunossay (NEN) and an enzymatic activity method (duPont). The upper limit for the TANDEM assay on nondiseased male patients was found to be 2.0 Mg/L. Based on this upper limit of normal, the diagnostic sensitivity of the method for all cases of prostatic carcinoma was 60%. We could not distinguish the enzyme released in abnormal amounts due

Table 2. Oligoclonal Bands in Cerebrospinal Fluid and the Clinical Likelihood of Multiple Sclerosis

<table>
<thead>
<tr>
<th>Clinical Impression (Multiple Sclerosis)</th>
<th>Number of Cases</th>
<th>Number of Cases with Oligoclonal Bands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not present</td>
<td>56</td>
<td>1</td>
</tr>
<tr>
<td>Unlikely</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Possible</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Probable</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Definite</td>
<td>13</td>
<td>12</td>
</tr>
</tbody>
</table>

help to clarify the meaning of the positive and negative assay results that were obtained.

The principal advantage of analytical disc gel electrophoresis on polyacrylamide gels as a method of demonstrating oligoclonal bands of IgG in cerebrospinal fluid is that unconcentrated samples as small as 200 µL can be assayed. The two-step method described in this paper exhibits sensitivity and specificity for multiple sclerosis comparable to the levels reported for electrophoresis in agarose and isoelectric focusing and also compares favorably to these other procedures with regard to the degree of difficulty and the time required.

Acknowledgment. We thank the neurologists of Christchurch, Dunedin, and Wellington Hospitals for providing clinical information on the patients. Mrs. J. Mooney typed the manuscript.

References

to benign prostatic hypertrophy and certain nonprostatic malignant diseases from that of prostatic carcinoma. The diagnostic specificity was calculated at 95%. For the clinically undetectable Stage I disease, sensitivity was 44% (four abnormal values out of nine cases). The TANDEM procedure is simple to use and reproducible. (Key words: Monoclonal antibody; Immunoradiometric assay; Prostatic acid phosphatase) Am J Clin Pathol 1983; 79: 114-119

THE ASSOCIATION of raised serum prostatic acid phosphatase (PAP; orthophosphoric monoester phosphohydrolase; EC 3.1.3.2) and carcinoma of the prostate has resulted in the development of many different methods for the measurement of this enzyme since the relationship was first described in 1938. The original colorimetric assays have recently been succeeded by immunologic ones, radioimmunoassay (RIA) in particular, with claims of improved specificity and sensitivity. However, the RIA procedures do have certain disadvantages, some of which relate to the nature of the antibodies that, until recently, have always been polyclonal, some to problems with purification and radiolabeling of the antigen, and some to the fact that competitive binding is the usual mechanism for the assay. We report the evaluation of a new, solid-phase, immunoradiometric method for the determination of PAP, which involves the simple “sandwiching” of this antigen between two monoclonal antibodies, with the one in solution carrying an 125I-radiolabel. This TANDEM assay is compared with an automated enzymatic colorimetric method and a competitive-binding, polyclonal RIA on a patient population with and without prostatic carcinoma.

Materials and Methods

Reagents

The reagent kits for the TANDEM-PAP assay were obtained from Hybritech Inc. (San Diego, CA) and contained the following materials: (1) antibodies—these were raised in mice after immunization with PAP, and their spleen cells were then harvested and fused with myeloma cell lines. The resulting hybridomas were selected according to their ability to bind 125I-labeled PAP and secretion of antibodies with good affinities. After subcloning, they were implanted into mouse peritoneum, and the monoclonal antibodies produced were obtained from ascitic fluid. Two different monoclonal antibodies were made to two different antigenic sites on the PAP molecule, solid-phase antibody—5/16-inch beads coated with monoclonal mouse IgG anti-PAP and labeled antibody—125I-labeled monoclonal mouse IgG anti-PAP in buffered saline containing human serum and 0.1% sodium azide; (2) PAP calibrators—lyophilized, containing human serum and sodium azide. The zero calibrator also serves as a specimen diluent, and the values of the five others were 1, 5, 10, 20, and 30 μg/L; (3) wash concentrate, 18 mL, containing 0.3% sodium azide; and (4) four controls for the assay, which were not lyophilized, with PAP values of 0, 2, 5, and 15 μg/L.

The source of PAP for both calibrators and controls was unpurified human seminal fluid.

Methods

The principle of this TANDEM (or sandwich) immunoradiometric assay is outlined in Figure 1.

The calibrators were reconstituted with distilled water and the wash concentrate was diluted to 500 mL with distilled water. One antibody-coated bead was dispensed into each tube using forceps, following by 200 μL of the appropriate calibrator, control, or test sample and 100 μL of 125I-labeled antibody tracer. All the tests were run in duplicate and the tubes were incubated for approximately 20 hours at 20–25°C. Two milliliters of wash solution were added to each tube and then aspirated;
this procedure was repeated one more time before placing all tubes in a gamma counter and determining the counts per minute (cpm) of each tube. A calibration curve was constructed on linear graph paper by plotting the cpm for each calibrator against the corresponding concentration of PAP. A best-fit smooth curve is drawn through the points, or the curve can be drawn with a plotter using a French curve fit.

Samples also were analyzed by the Rianen procedure (New England Nuclear Medical Diagnostics, Billerica, MA, which has been in routine use in our laboratory for the past two years. This is a 4-hour competitive binding RIA, using 125I-labeled PAP as the antigen, rabbit anti-PAP antiserum as first antibody, and sheep anti-rabbit antiserum as second precipitating antibody. The calibration curve is constructed on semi-logarithmic graph paper plotting %B/Bo for each calibrator against the corresponding concentration of PAP in µg/L.

Most of the samples were also analyzed by the enzymatic method used on the duPont aca III (duPont, Wilmington, DE). This automated procedure employs thymolphthalein monophosphate as substrate, which is hydrolyzed by PAP to thymolphthalein; the reaction is terminated after 3.7 minutes with sodium hydroxide. Absorbance at 600 nanometers is due to thymolphthalein formed in proportion to the PAP activity in the sample, and is reported as U/L. A second pack is used to provide a serum blank correction.

Patients

Serum samples were obtained from patients at the Veterans Administration Medical Center, San Diego, CA. These 201 specimens were sent to the clinical laboratory for serum PAP values.

An aliquot of 1 mL was acidified with 10 µL of glacial acetic acid for analysis by the aca method. The rest of the serum was stored at −20°C for up to one week before testing by the immunoassays. No samples were frozen and thawed more than once.

Clinical histories were used to divide the patients into the following groups: (1) normals—54. These patients were men aged 30–90 years with normal prostates on clinical examination and no evidence of any malignant disease; (2) carcinoma of the prostate—67. Diseases were confirmed histologically in all cases; (3) benign prostatic hyperplasia (BPH)—36; (4) malignant disease other than prostatic—40; (5) women—4.

Results

The following analytic parameters were noted for the TANDEM PAP assay: analytic range without sample dilution, 0–30 µg/L; 700 cpm for the O standard (solid-phase antibody plus labeled antibody) and 30,000 cpm for the 30 µg/L standard; total counts—110,000 cpm. See Figure 2 for a typical standard curve. The RIANEN method compares as follows: analytic range without sample dilution, 0–50 µg/L; O standard—14,000 cpm, and 50 µg/L—3,600 cpm; total counts—35,000 cpm; nonspecific binding (omission of anti-PAP to O standard)—1,800 cpm.

Day-to-day precision was determined for the three methods on normal and abnormal lyophilized control sera. Results are listed for mean, standard deviation, and coefficient of variation: TANDEM (µg/L)—2.49, 0.33, 13% and 15.8, 1.88, 12%; NEN (µg/L)—2.36, 0.51, 22% and 11.8, 1.13, 10%; aca (U/L)—0.82, 0.08, 10% and 4.56, 0.28, 6.1%.

The normal range for the TANDEM PAP assay assessed by nonparametric methods and derived from the 54 normal adult male patients is 0–2.0 µg/L. The normal ranges for the other two methods, as published in the manufacturer’s literature, are 0–3 µg/L for the NEN and 0–0.85 U/L for the aca. The four females had detectable levels of serum PAP within the normal range by each method.

Figure 3 shows the comparison between the TANDEM and NEN methods on 132 patient samples. The calculated linear regression equation for this data is Y(TANDEM) = −0.9 µg/L + 0.777 × (NEN), with a correlation coefficient of 0.981. Figure 4 shows the com-
FIG. 3. Correlation graph of PAP results by TANDEM and NEN-RIA methods. (Line drawn according to linear regression equation; overlapping points not shown).

FIG. 4. Correlation graph of PAP results by TANDEM and aca methods. (Line drawn according to linear regression equation; overlapping points not shown.)

Comparison between the TANDEM and aca results on 113 samples. The data yielded $Y(\text{TANDEM}) = -2.56 \mu g/L + 7.76X (\text{aca})$, with a correlation coefficient of 0.886. Since these two PAP methods use different units, the linear regression equation would not be expected to have a slope of unity. The majority of the samples used were the same for both comparisons, but fewer samples were run by the aca than by the radioassays.

The results of the clinical studies are summarized in Table 1. The diagnostic sensitivity figures were derived using the number of patients with carcinoma of the prostate having levels of PAP above the normal range as a per cent of the total number with that disease. The specificity calculation (number of patients without carcinoma of the prostate having PAP levels within the normal range as a per cent of all such patients) includes those patients with BPH, nonprostatic malignant disease, and those classified as normal. Four patients in this group with hyperlipidemia showed PAP levels between 3.0 and 10.0 \( \mu g/L \) by NEN but were within the normal range by the other two methods. The efficiency of the test expresses the sum of the patients with true-positive and true-negative results as a per cent of all the patients tested. Positive predictive values (true positives as a per
Table 1. Summary of Calculated Diagnostic Indices of PAP Assays

<table>
<thead>
<tr>
<th></th>
<th>TANDEM†</th>
<th>NEN†</th>
<th>aca‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>60</td>
<td>55</td>
<td>53</td>
</tr>
<tr>
<td>Specificity</td>
<td>95</td>
<td>90</td>
<td>97</td>
</tr>
<tr>
<td>Efficiency</td>
<td>83</td>
<td>78</td>
<td>82</td>
</tr>
<tr>
<td>Predictive value of a positive test</td>
<td>85</td>
<td>73</td>
<td>88</td>
</tr>
<tr>
<td>Predictive value of a negative test</td>
<td>83</td>
<td>75</td>
<td>81</td>
</tr>
</tbody>
</table>

* See text for method of calculation of indices.
†† Includes data from 67† or 40‡ patients with prostatic carcinoma and 134† or 81‡ without.

Table 2 classifies the prostatic carcinoma patients by stage12 and presents the sensitivity for each stage.

In the group of patients with neoplastic disease other than prostatic, six had PAP levels above the normal range by one or more of the assays. Two of these patients had myeloid leukemia, two had non-Hodgkin’s lymphomas, and, of the other two, one had a carcinoid tumor of the lung and the other had a metastatic adenocarcinoma of the lung. The highest values were from the latter patient and were 17.2 µg/L, 22.4 µg/L, and 2.7 U/L by TANDEM, NEN, and aca, respectively. The other patients had values within twice the upper limit of normal by each method. Some values slightly above the normal range were found by each method for patients with BPH. In this group, the per cent of positive results by TANDEM, NEN, and aca was 5.6, 5.6, and 4.0, respectively.

Discussion

It appears from the regression equations and correlation coefficients that the TANDEM assay compares well with both the NEN and aca methods, and the precision of the technic is satisfactory.

Table 2. Sensitivity of Three PAP Methods Based on Prostatic Carcinoma Stages

<table>
<thead>
<tr>
<th>Stage</th>
<th>TANDEM</th>
<th>NEN</th>
<th>aca</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Per Cent of Positive Cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>44 (4/9)*</td>
<td>22 (2/9)</td>
<td>25 (2/8)</td>
</tr>
<tr>
<td>2</td>
<td>29 (4/14)</td>
<td>21 (3/14)</td>
<td>17 (1/6)</td>
</tr>
<tr>
<td>3</td>
<td>50 (5/10)</td>
<td>50 (5/10)</td>
<td>38 (3/8)</td>
</tr>
<tr>
<td>4</td>
<td>79 (27/34)</td>
<td>79 (27/34)</td>
<td>83 (15/18)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are number of cases with PAP results greater than the normal range out of the total number tested for each stage.

The monoclonal antibody is homogeneous as it is secreted by cells derived from, and identical to, the original hybridoma. It will bind, therefore, only with one binding site on a specific antigen. A polyclonal population of antibodies is heterogeneous in that they will bind with different sites on the antigen and may well cross-react with other similar antigens.† For this reason, nonspecific binding, a recognized problem with RIAs, will be reduced in a monoclonal assay. The fact that the normal range by TANDEM is only up to 2.0 µg/L as opposed to 3.0 µg/L by NEN bears this out, with the NEN range taking into account higher nonspecific binding.

The combination of unique antibody specificity and very high analytic sensitivity of radioisotope detection should detect unequivocally any prostatic acid phosphatase protein circulating in the blood. Unfortunately, this protein moiety is not unique to prostatic carcinoma and may appear frequently in cases of BPH and occasionally in certain other malignant diseases. This limitation on diagnostic specificity (appearance of abnormally high PAP values in nonprostatic carcinoma patients) is observed here, as well as in other investigations.2,5,7 PAP was found in the serum of all four women by each assay and has been detected previously in female urine.11 This suggests that either PAP is not, in fact, prostate-specific and has an alternative tissue source in women, or that the assays are not sufficiently specific to detect only the prostatic isoenzyme of acid phosphatase.

In terms of diagnosis, an enzyme assay has its major utility at stage 1, because in all other stages, the tumor is detectable on clinical examination.12 Achievement of good sensitivity when the disease is occult has been the stimulus for development of a large number of different methods for PAP measurement. However, even with this new monoclonal antibody assay, the figure is still less than 50%. Presumably, the amount of PAP secreted by an early collection of malignant cells is very small and insufficient to increase the circulating concentration above the normal range. Thus, no assay, however sensitive analytically, will be able to identify the disease under these circumstances.

Given the limitations already noted, the TANDEM procedure provides a technically simple, reliable, and more specific biochemical determination to be used in conjunction with clinical findings in the diagnosis and therapy of prostatic disease. In particular, the nearly linear standard curve provides excellent discrimination of very low, normal, and slightly raised PAP results.

Acknowledgments. The authors wish to thank Hybritech, Inc., for providing the TANDEM PAP kits used in this investigation.
Intravenous fluids were infused into the forearms of 18 volunteers. Baseline hematologic and serum biochemical profiles were obtained from each volunteer prior to starting the IV. After the intravenous fluids had infused for 30 minutes, blood was drawn from the opposite arm, and above and below the IV in the same arm. The intravenous fluids were then stopped, and after waiting two minutes, another blood sample was drawn from the IV needle. The deviation from the baseline value was determined for each analyte by sampling site for each volunteer, and the mean deviation was calculated for each analyte from each sample site. Drawing blood from above the infusing IV line resulted in a dilutional effect for most of the analytes. Most analytes were not affected when blood was drawn from the other sites. Serum glucose and phosphorus had mean deviations greater than two standard deviations from the baseline, regardless of where they were drawn. Serum glucose was the only analyte with values higher than the baseline values. We recommend that serum biochemical and hematologic profiles not be drawn from the opposite arm or below the IV while it is infusing or out of the IV needle after the intravenous fluids have been stopped for two minutes. (Key words: Serum biochemical and hematologic profiles; Intravenous fluids) Am J Clin Pathol 1983; 79: 119-121

WHERE SHOULD BLOOD be drawn when a patient is receiving intravenous fluids? Can blood be drawn from above the IV without altering the results? These become important questions when blood cannot be drawn from the opposite arm in cases of recent mastectomy, amputation or when the opposite arm has no patent vein or is covered with bandages. We undertook a study to evaluate this problem using 18 healthy volunteers.

Materials and Methods

Intravenous fluids were infused into the forearm of 18 volunteers, 10 women and eight men, ages 20 to 45 years using an 18-gauge, 1½-inch Jelco® needle. All the volunteers believed themselves to be healthy. Blood for baseline hematologic and serum biochemical profiles was drawn from the IV needle prior to starting the intravenous fluids. Nine patients received 5% dextrose in water (D5W) and nine received 5% dextrose in ½ normal saline solution (D5W in ½ NS). The rate of intravenous fluid flow was approximately 125 mL/hour. After intravenous fluids were infused for 30 minutes and while the IV was still infusing, blood was drawn from the opposite arm, and above and below the IV in the same arm with...