Measurements of Serum γ-Seminoprotein and Prostate Specific Antigen Evaluated for Monitoring Carcinoma of the Prostate

Jill K. Siddall,1 Sugandh D. Shetty,2 and Edward H. Cooper1

We have compared the concentrations in serum of γ-seminoprotein (γ-SM) and prostate specific antigen (PSA), two antigens of prostatic origin that are synthesized independently of prostatic acid phosphatase (PAP, EC 3.1.3.2), to assess their potential in monitoring prostatic cancer. At presentation, 27/30 (90%) patients with metastases had a PSA concentration >10 ng/mL and 29/30 (97%) a γ-SM concentration >10 ng/mL; 21/61 (34%) with disease but without metastases had an abnormal content of PSA, and 23/61 (38%) an abnormal γ-SM. Concentrations of PSA and γ-SM were significantly correlated (r = 0.68, p <0.001). In 20 patients without metastases followed longitudinally, the median concentrations of γ-SM, PSA, and PAP in the 13 patients who developed bony metastases or showed signs of local spreading of the tumor were 58 ng/mL, 34 ng/mL, and 2.1 U/L, respectively. The corresponding median values in the seven patients who remained clinically stable were 2.5 and 3.9 ng/mL, and 2.3 U/L. We conclude that either PSA or γ-SM can warn of disease progression when PAP activities are still within normal limits.

Additional Keyphrases: cancer • monoclonal antibodies • immunoenzymometric assay • immunoradiometric assay • prostatic acid phosphatase compared

Two independent lines of research have developed in the search for specific antigens that can be used to monitor prostatic cancer. In one, Harai et al. (1) isolated two glycoproteins from seminal fluid, γ-seminoprotein (γ-SM) and β-microseminoprotein, having molecular masses of 23 000 and 10 400 Da, respectively.2 Harai then developed a sandwich-type immunoassay involving passive hemaggulination to detect seminoprotein (2), and used this in forensic medicine to verify the presence of seminal stains (2, 3). A noncompetitive polyclonal antibody radioimmunoassay of the two glycoproteins was developed in 1982 by Okabe and Eto (4), who found concentrations of serum γ-SM >1.93 ng/mL in 4% of normal controls, 41% of nonprostate cancer patients, 87% of the patients with benign prostatic hyperplasia, and 91% of prostate cancer patients. They concluded that γ-SM was a potential marker of prostatic cancer, and was a better indicator than β-microseminoprotein (5). Several monoclonal antibodies against γ-SM have been raised and a monoclonal sandwich-type enzyme immunoassay has been developed (6, 7). In the present study we have compared the performance of two commercial enzyme immunoasays for γ-SM, developed in Japan, based on the use of different monoclonal antibodies.

1 In a separate approach, Wang et al. (8) purified an antigen from the prostate gland that was distinct from prostatic acid phosphatase (PAP, EC 3.1.3.2), which they called prostate specific antigen (PSA, sometimes abbreviated PA in the literature). This antigen (molecular mass 34 000 Da, pi 6.9) is confined to the cytoplasm of the acinar cells and the ductal epithelium of the prostate gland. PSA has been detected by immunohistochemical staining in all normal, benign hyperplastic, and cancerous prostate specimens, but not in other normal or malignant tissues. Several studies of the role of serum PSA as a marker in prostatic cancer have been published (8–10).

Here we have examined the concentrations of γ-SM in serum from patients with prostatic cancer and various controls and compared them with the corresponding concentrations of PSA. For our longitudinal studies we selected patients with untreated carcinoma of the prostate, a group in which PSA appeared to be a valuable marker of early progression of malignancy (11).

Materials and Methods

Patients

Twenty patients with prostatic cancer localized to the gland (M0) were followed for as long as 43 months (median 22 months). All patients were receiving no treatment at the start of the investigation; 13 were eventually treated because their cancer progressed. An additional 71 patients with carcinoma of the prostate were investigated before treatment, to investigate the relationship between the concentrations of γ-SM, PSA, and the stage of the disease. The cancers were staged T0-4N0M0-1 according to the system of the Union International Contre le Cancer and the histology graded G1–G3: T0-4 describes the stage of the tumor, Nx means lymph nodes status unknown, M0 = no distant metastases, G1 = distant metastases, G1 = well differentiated, G2 = moderately differentiated, and G3 = poorly differentiated (12).

We also examined two control groups: 30 healthy male blood donors and 25 patients with benign prostatic hyperplasia.

Serum samples were separated from blood within 2–3 h of collection and stored at −20 °C until assay. We acidified an aliquot of each serum before measuring PAP enzymatic activity with a Boehringer (Mannheim, P.R.G.) acid phosphatase kit (13) (normal values <3.1 U/L).

We measured γ-SM with an enzyme immunoassay kit (Chugai Pharmaceutical Co., Tokyo, Japan), which contained type 13A6 monoclonal antibody. Mouse monoclonal antibodies directed against γ-SM form the solid phase, and a rabbit antibody labeled with horseradish peroxidase (EC 1.11.1.7) is used as the second antibody in the sandwich. Absorbance at 492 nm is used to construct the standard curve. We also examined a second Chugai assay for γ-SM, in which type CK monoclonal antibody acts as the first antibody.
To measure PSA, we used the Tandem® PSA-RIA (Hybritech, San Diego, CA), according to the manufacturer's instructions. A mouse monoclonal antibody to human PSA is coated onto a plastic bead (solid phase) and another radiolabeled mouse monoclonal antibody directed against a distinctly different antigenic site on the PSA molecule is the second antibody in the sandwich.

Results

The mean ± SD concentration of serum γ-SM determined with the 13A6 assay for 30 normal male controls (median age, 44 years) was 5.4 ± 4.8 ng/mL; corresponding PSA concentrations were 2.24 ± 2.10 ng/mL. Serum γ-SM values in 26 patients with benign prostatic hyperplasia was 22.1 ± 42.9 ng/mL; the corresponding results obtained with the type CK kit were 19.7 ± 29.1 ng/mL.

The coefficient of variation (CV) for γ-SM (13A6) for two sera with mean concentrations of 75.6 and 168 ng/mL was 3.9% and 3.8%, respectively, within assay (n = 20) and 10.4% and 4.4%, respectively, between assay (n = 20).

A previous evaluation (11) of the reproducibility of PSA in our laboratory for three sera with mean concentrations of 3.1, 6.8, and 35.1 ng/mL gave respective CVs of 3.2%, 2.1%, and 1.3% (within assay) and 6.1%, 3.2%, and 3.1% (between assay).

Data at Presentation

The distribution of γ-SM and PSA values in carcinoma of the prostate according to histological grade and the presence or absence of metastases in patients at presentation is shown in Figure 1. The same data stratified for T stage are shown in Table 1. The relationship between PSA and γ-SM at presentation (Figure 2) is highly significant (r = 0.68, p <0.001). The correlation remains significant for the subsets of patients: for M0 patients (n = 61), r = 0.75 and p <0.001, for M1 patients (n = 30), r = 0.60 and p <0.001.

Longitudinal Data

The 20 patients presenting with tumors localized to the prostate gland and observed without treatment were followed longitudinally. The median and range for γ-SM, PSA, and PAP at presentation and in the last sample in patients without progression—or at the time of progression, if this occurred—are shown in Table 2.

The median values of the three indicators in 13 patients who developed bony metastases or showed signs of local extension of the tumor within 38 months (median 21 months) were 58 ng/mL, 34 ng/mL, and 2.1 U/L for γ-SM, PSA, and PAP, respectively. By contrast the median values in the seven patients who remained stable clinically were 2.5 ng/mL, 3.9 ng/mL, and 2.3 U/L. In this small series, the initial concentrations of γ-SM and PSA at presentation were higher in the group that progressed than in the stable group. In such a small group, however, a test of significance would be meaningless.

Table 1. Concentrations of PSA and γ-SM in Serum of Prostatic Cancer Patients at Presentation

<table>
<thead>
<tr>
<th>Stage</th>
<th>Analyte</th>
<th>&lt;2</th>
<th>2–10</th>
<th>11–100</th>
<th>&gt;100</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0–M0</td>
<td>PSA</td>
<td>15*</td>
<td>19</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>γ-SM</td>
<td>7</td>
<td>26</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>T2–M0</td>
<td>PSA</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>γ-SM</td>
<td>1</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>T2–M1</td>
<td>PSA</td>
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<td>3</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>γ-SM</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>22</td>
</tr>
</tbody>
</table>

*No. of patients.

Fig. 1. Distribution of γ-SM (13A6 assay) concentrations (left) and prostate-specific antigen concentrations (right) at the time the patient entered the hospital, according to histological grade.
Table 2. Changes in γ-SM, PSA, and PAP
Concentrations in Untreated Patients with Prostatic Cancer

<table>
<thead>
<tr>
<th>Concordance and range</th>
<th>Median concn (and range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-SM, ng/mL</td>
<td>PSA, ng/mL</td>
</tr>
<tr>
<td>Stable disease (n = 7)</td>
<td></td>
</tr>
<tr>
<td>At presentation</td>
<td>1.2</td>
</tr>
<tr>
<td>Last sample*</td>
<td>(0.0–9.5)</td>
</tr>
<tr>
<td>Progressive disease</td>
<td></td>
</tr>
<tr>
<td>At presentation (n = 13)</td>
<td>15.0</td>
</tr>
<tr>
<td>At clinical progressionb</td>
<td>(2.6–165)</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(2.6–430)</td>
</tr>
</tbody>
</table>

*14–43 months after presentation, median 36 months. 24–38 months after presentation, median 21 months.

Discussion

The search for improved tumor markers for monitoring prostatic cancer has resulted in two major changes in the commercial tests currently available. The first, the development of several immunological assays for PAP, is now considered to have small advantages over the enzymatic measurements of PAP. Mainly, the increased reproducibility of the immunological assays means that changes close to the upper limit of normal can be interpreted with increased confidence (14, 15).

The discovery in the prostate and seminal fluid of glycoprotein antigens derived specifically from prostatic tissue has opened up a new chapter in tumor markers for prostatic cancer. Western investigators have been slow to follow the line of research developed by Japanese workers, whereby tests for γ-SM have evolved from the special needs of forensic medicine to monitoring of prostatic cancer. It is now clear that concentrations of γ-SM in serum are increased in metastatic prostatic cancer, although the various assays—which include radioimmunoassays (4) and enzyme-linked immunosorbent assays (16, 17)—show some differences in their normal values and range of response.

In the United States, the National Prostatic Cancer Project has led a multicenter clinical evaluation of the measurement of serum PSA as a marker of prostatic cancer (8, 10, 18). Tests for PSA have become commercially available, and its value in monitoring treatment has been confirmed (11), especially in the progression from nonmetastatic to metastatic disease (18).

There is still some confusion as to whether the two antigens γ-SM (M, 23 000) and PSA (M, 34 000; 8) are the same or at least share common epitopes. The glycan structures of γ-SM have been analyzed in detail (20), and immunohistochemical studies of the distribution of immunoreactive γ-SM and PSA show them to be closely related in carcinoma of the prostate (20). Polyacrylamide gel electrophoresis of purified γ-SM produced six bands, one of which coincided with purified PA (7).

Our clinical studies of patients indicated that serum concentrations of γ-SM and PSA are correlated at presentation (r = 0.68), and remain concordant in the longitudinal studies. The γ-SM values were generally higher than the corresponding PSA concentrations, although the difference was only 1–2 ng/mL when the samples were in the low range. The range of normal values we found in the γ-SM (13A6) assay, 5.4 ± 4.8 ng/mL, was higher than reported for Japanese males (<4 ng/mL, according to the kit manufacturer).

Given the close similarity of the antigens, it is no unexpected that either γ-SM or PSA can indicate disease progression; however, either is useful when the PAP values are still within normal limits (Table 2).

In terms of convenience for the user, the double monoclonal assay of PSA involves fewer steps in the assay procedure than the γ-SM method, and probably affords better accuracy at the lower end of the standard curve. Clinically, however, minor deviations from the upper limits of normal are unimportant, and both tests are more sensitive than enzymatic measurement of PAP. As shown in the presentation data, γ-SM is unsuitable as a diagnostic agent and should not be used for screening. The same applies to PSA (11).

The history of the inappropriate use of immunochromic assays of PAP for screening is a salutary lesson of what can happen if conclusions are drawn prematurely about the diagnostic efficacy of a particular test. Only studies over periods commensurate with the natural history of the evolution of M0 disease, in which about 30% of patients will progress in five years, will convince urologists as to which tests can best aid in the management of their patients.

J.K.S. and E.H.C. are supported by the Yorkshire Cancer Research Campaign. We are grateful to Hybritech for supplying the PSA kits, to Chugai Pharmaceuticals, Japan, for supplying the γ-SM kits used in this evaluation, and to Drs. John Bruni and R. Wakebaysahi for their helpful advice. We thank Dr. F.R. Teasdale for the routine assays of PAP.

References


