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Prostate-specific Antigen: Problems in Analysis

Atilla Turkes, Jayne P. Nott and Keith Griffiths

We have compared an "in-house" Tenovus Institute prostate-specific antigen (PSA) assay with four different commercial kits (ELSA-PSA, IRMA-Count PSA, PROS-CHECK PSA and TANDEM-R PSA) that are available in the UK. There was only good correlation and linear regression parameters between the in-house assay and one of the kit methods. The difference in values for the same sample ranged from 2 to 100-fold. These discrepancies are due, in part, to the specificity of the polyclonal and monoclonal antibodies used in the procedures and the differing "hook effects" caused by the binding capacity of the antibody pairs in the immunometric assays. Discrepancies will, however, result from the differing potencies of the standards used for the calibration curves. This data highlights the urgency for the introduction of an internationally accepted reference standard for PSA.
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INTRODUCTION

PROSTATE-SPECIFIC ANTIGEN (PSA) is a recently isolated glycoprotein of prostatic origin [1] secreted by the epithelial cells into the seminal plasma. It is a serine protease, similar to kallikrein and thought to be responsible for the liquefaction of seminal coagulum [2, 3]. It is found on the endoplasmic reticulum and also in cytoplasmic vesicles and secretory granules of the normal, benign hypertropic and neoplastic prostate epithelial cells. The organ specific nature of this antigen stimulated research into its potential as a tumour marker and its determination has proved to be useful, not only for immunohistological identification of malignant tumours of prostatic origin [4, 5], but also for the determination of serum levels in the management of patients with prostatic cancer [6–8]. Comparative studies have also revealed that circulating PSA concentrations provide more information than prostatic acid phosphatase for the detection of current or future status of the disease [9–11].

The value of the determination of PSA in serum is becoming well established in the routine practice of general practitioners and urology clinics for the evaluation of patients with prostatic disease. The majority of these determinations are performed by commercially available immunoassay kits. As part of the Institute's prostate cancer research program, we have developed an "in-house" chemiluminometric assay (CLIA) for the determination of PSA in serum incorporating acridinium ester-labelled monoclonal antibody. We have compared the in-house CLIA with four different commercially available kits that are marketed in the UK.

Correspondence to A. Turkes.
The authors are at the Tenovus Institute for Cancer Research, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XX, U.K.
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MATERIALS AND METHODS

Patients and samples

Patients with previously untreated metastatic carcinoma of the prostate participated in a phase III randomised trial [12, 13]. All patients had histologically proven carcinoma of the prostate with metastases and received first line endocrine treatment of either orchidectomy or a monthly injection of a depot luteinising hormone-releasing hormone analogue (goserelin). Serum samples were collected from patients at different stages of their treatment and stored at −20°C or lower until assayed.

Purification of PSA

PSA was purified from seminal plasma by a procedure similar to that described by Wang and his colleagues [14]. A pool of seminal plasma (40 ml) was ammonium sulphate fractionated, dialysed and applied to an ion-exchange column (1.6 × 40 cm) packed with DEAE Sepharose CL-6B (Pharmacia-LKB Biotechnology). The PSA was eluted from the column by a linear salt (NaCl) gradient and fractions containing PSA were pooled, concentrated and subjected to gel exclusion column (2.6 × 90 cm) chromatography on Sephacryl S-200 (Pharmacia–LKB Biotechnology). Following elution of the column with Tris-HCl (0.01 mol/l, pH 8) buffer, fractions containing PSA were pooled, concentrated and applied to a DEAE Sepharose CL-6B column. This time PSA was eluted by pH gradient and PSA fractions, following further gel exclusion chromatography on Sephacryl S-200, were pooled and the final protein concentration was determined (BCA protein assay reagent, Pierce). Each purification run yielded 3–5 mg PSA and the purity of the protein was >95% on polyacrylamide gel electrophoresis.

Antisera

Sheep, each 8–10 months old, were immunised by multiple injections of purified PSA (200 μg). The primary immunisation

was initiated by subcutaneous injection, to the upper quarter of the hind legs, of antigen in complete Freund's adjuvant (Difco Laboratories). Later booster injections were administered subcutaneously with incomplete Freund's adjuvant (Sigma) at monthly intervals. Test bleeds were taken 2 weeks after the third injection to determine PSA antibodies.

All sheep produced adequate titres after the third booster injection as assessed by ELISA on microtitre plates. The antiserum (code: Y42) used in the CLIA was precipitated with sodium sulphate for the preparation of immunoglobulin rich fractions and covalently coupled to cyanogen bromide-activated cellulose beads, following a previously published procedure [15]. The monoclonal PSA antibody (Hybritech) was labelled with active acridinium ester [16] and used as tracer in the CLIA.

Standards

Standards used in the CLIA were prepared in male serum following the absorption of endogenous PSA by affinity chromatography. A serum pool (500 ml) was incubated with an excess concentration of cellulose-coupled PSA antibody for 24 h at 4°C. Following centrifugation, the supernatant was filtered and spiked with purified PSA to give appropriate concentrations of standards used in the CLIA.

Immunoassays

The in-house CLIA incorporated a polyclonal solid-phase antibody and an acridinium ester-labelled monoclonal antibody. The assay was performed by incubating standards and samples together with solid-phase and labelled antibodies for 2 h at room temperature followed by washing the solid-phase and measuring the chemiluminescent activity in a luminometer (Berthold) associated with the bound complex. The sensitivity of the assay was 0.12 ng/ml and the estimated intra-assay and interassay coefficients of variation, over the working range, were 4.8–8.9% and 3.7–10.9%, respectively.

ELSA-PSA (CIS International) is an immunometric method featuring two monoclonal antibodies with different antigenic determinants. The first monoclonal antibody is coated on the polypropylene fins, which are inserted into the bottom of the tubes and the second antibody is labelled with ^{125}I-NaI and used as tracer in the assay. IRMA-Count PSA (Diagnostic Product Corporation) is an amplified immunoradiometric assay based on ligand-coated tubes and three liquid monoclonal, anti-PSA antibodies, one ^{125}I-labelled, the other two linked to a ligand. PSA in the standard, or in the patient sample, is captured between the monoclonals, in a reaction proceeding with liquid-phase kinetics. Separation is then achieved by the ligand-coated tube/antiligand bridge method.

TANDEM-R PSA (Hybritech) is also a solid-phase immunometric assay incorporating two monoclonal antibodies directed against distinctly different antigenic sites on the PSA molecule. Solid-phase is prepared by coating plastic beads with one monoclonal antibody and the other antibody is used to prepare radio-iodinated tracer. PROS-CHECK PSA (Yang Laboratories) on the other hand is a double antibody radioimmunoassay based on the competitive binding assay principles. PSA, from standards and samples, is simultaneously reacted with a rabbit polyclonal antibody and ^{125}I-labelled PSA. Separation of antibody-bound and free tracer is affected by a second precipitating antibody (goat anti-rabbit). In this type of assay the antibody-bound antigen is inversely proportional to the amount of antigen present in the standards or samples, whereas in the immunometric assays, the antibody-bound antigen is directly proportional to the antigen present in the standards or patient samples.

Table 1. PSA concentrations in samples from prostate cancer patients determined by five different assays

Sample	CLIA	ELSA	IRMA-Count	PROS-CHECK	TANDEM-R
1	1.5	5.3	6.7	5.9	1.7
2	1.8	3.4	0.4	2.8	1.9
3	2.0	1.9	2.6	1.1	3.9
4	4.1	5.4	3.4	4.8	4.1
5	4.2	7.5	6.9	8.4	3.9
6	4.4	3.2	6.5	3.0	4.7
7	6.3	8.0	6.1	5.1	6.9
8	6.4	8.3	5.9	4.7	5.2
9	11.7	27.1	23.3	30.0	13.0
10	14.9	26.2	27.6	16.0	11.3
11	21.1	56.3	63.1	38.4	36.1
12	24.6	69.9	56.2	82.9	26.6
13	45.6	115.8	109.7	98.0	62.3
14	122.0	190.3	156.6	195.0	129.6
15	137.1	238.6	135.5	251.0	112.0
16	141.5	310.5	185.7	214.0	176.5
17	206.8	332.1	185.2	294.0	225.5
18	249.4	394.3	382.7	246.0	201.5
19	328.7	650.8	538.6	290.0	287.1
20	503.4	905.8	352.4	285.0	519.4
21	561.0	1256.8	165.2	471.0	658.8
22	892.8	1404.4	1221.1	517.0	965.2
23	2114.0	14878.0	52.0	555.0	2300.0
24	2340.0	21024.0	31.2	373	2592.0

All assays were performed according to the manufacturers' instructions and the results were calculated using MultiCalc (Pharmacia–LKB) immunoassay software package interfaced to a NE 1600 gamma counter (NE Technology).

RESULTS

Serum samples collected from patients with prostate cancer during different stages of their treatment were assayed by the five methods and the results are presented in Table 1. There was a good correlation ($r > 0.96$) between the CLIA and TANDEM-R PSA assays. The regression analysis shows that the slope does not differ significantly from one ($m = 0.91$) or the intercepts from zero ($c = 6.38$ ng/ml). The correlation between CLIA and ELSA-PSA was also satisfactory ($r > 0.93$) but the regressing parameters were poor ($m = 0.12$; $c = 116.96$ ng/ml). There was little or no correlation between the results obtained by the in-house CLIA and the other two kit methods.

The calibration standards provided by the kits were assayed by CLIA as samples. The results determined were plotted against expected concentration values and presented in Fig. 1.

DISCUSSION

These data illustrate the variable numerical values of serum PSA concentrations which were determined by different assay procedures. It was suggested that these variations are partly due to the specificity of the polyclonal and monoclonal antibodies used in the immunoassay procedures [17]. Falsely low values, the result of the "hook-effect" obtained in some samples with immunometric assays, is another contributory factor for the

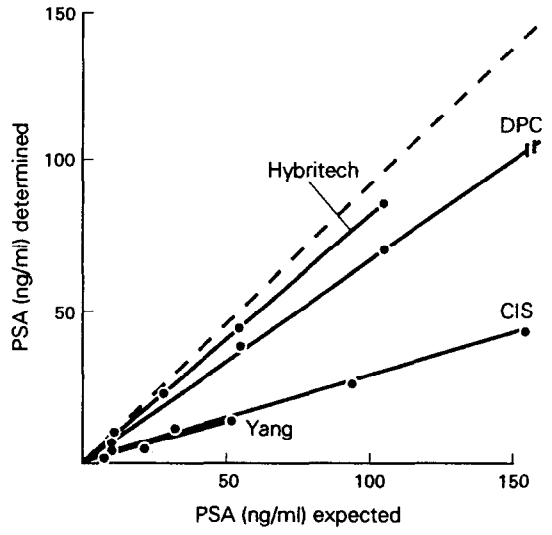

Fig. 1. Determination of PSA concentrations in kit standards by CLIA.

differences [18, 19]. It was also suggested that if female serum is used as the matrix for the preparation of PSA standards, the possibility of endogenous PSA antibodies, formed in the same manner to that of sperm antibodies [20], or PSA directly secreted by the female prostate [21], could cause variations in results. The inherent problem is however, as demonstrated in Fig. 1, the variation in the potencies of standards used for the calibration curves.

The literature suggests that the data on the usefulness of PSA in the clinical studies are accumulating with definitive statements such as ". . . a value of >20 ng/ml in patients with acute retention is highly indicative of prostate cancer" [17]: ". . . once a PSA level of 88 ng/ml was reached, there was an average time of less than two months before recurrence was clinically confirmed" [22]: ". . . serum PSA levels of >10 ng/ml at diagnosis, carried an increased risk of progression within two years" [9]. It is imperative that when such quite definitive statements are made, attention is also drawn to the particular method of measurement used to avoid misleading the readers into the assumption that universally accepted "cut-off points" exist, or certain "ranges" relate to the natural history of prostatic disease. Furthermore, the incompatibility of the methods may induce difficulties in long-term compilation of data or in the assessment of patients referred from one hospital to another and moreover they would create complications in epidemiological studies where data from multiple sources are to be evaluated. Such problems may be overcome by the introduction of international reference standards for PSA rather similar to that of other peptide hormones. This concept, in the long term, would induce the creation of a universal reagent.

Addendum—During the preparation of this manuscript, two more PSA kits, DELFIA Prostate Specific Antigen (Pharmacia-Wallac Oy) and ELSA-PSA 2 (CIS International), appeared on the market. We were informed by CIS that ELSA-PSA 2 is exactly the same as the method used in our studies except that the standards were calibrated against TANDEM-R PSA.

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