The Determination of Prostate-Specific Antigen (PSA) in Human Female Sera by Means of a Sensitive ELISA

Bestimmung des Prostata-spezifischen Antigens (PSA) in humanen weiblichen Seren unter Verwendung eines sensitiven ELISA

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Summary: The development of a sensitive sandwich ELISA for the determination of prostate-specific antigen in serum is reported. The assay makes use of the monoclonal antibodies mAB43A2 and mAB28A4. The analysis of 231 sera from female patients with the tentative diagnosis of breast cancer yielded in 31 cases PSA concentrations above the detection limit of the assay (0.08 µg/l). Serum PSA should therefore not further be regarded as an absolutely specific marker of prostatic tissue.

Keywords: Antibodies, Monoclonal; Enzyme-linked Immunosorbent Assay; Female; Prostate-specific Antigen/serum.

Zusammenfassung: Es wird über die Entwicklung eines sensitiven Sandwich-ELISA zur Bestimmung von Prostata-spezifischem Antigen (PSA) im Serum unter Verwendung der monoklonalen Antikörper mAB43A2 und mAB 28A4 berichtet. Bei Analyse von 231 Seren von Patientinnen mit der Verdachtsdiagnose Mammacarcinom fanden sich in 31 Fällen (13,4%) PSA-Konzentrationen oberhalb der Nachweisgrenze des ELISA (0,08 µg/l). PSA im Serum kann daher nicht weiter als ein absolut für Prostatagewebe spezifischer Marker angesehen werden.

Schlüsselwörter: Antikörper, Monoklonale; Enzyme-linked Immunosorbent Assay; Prostata-spezifisches Antigen/Serum; Weiblich.

The usefulness of prostate-specific antigen (PSA) as a marker of adenocarcinomas of the prostate has been amply demonstrated. In recent years, PSA determination in serum has become an important tool for monitoring of prostate carcinomas in patients under therapy.

For several reasons the development of PSA assays is relatively difficult. For example, PSA is present in sera in various forms, some of which are not well-characterized. It has been reported that PSA is present in sera predominantly as complex with α_1-antichymotrypsin and to lesser extent bound to other serum proteins such as α_1-macroglobulin and inter-α-trypsin inhibitor [1,2].

Another difficulty associated with PSA assays is the lack of a generally acceptable standard preparation. The various PSA subfractions in the standard preparations are possibly different from those in patient sera. These differences may lead to different results because monoclonal and/or polyclonal antibodies recognize PSA subfractions with different affinities [1, 2]. One way to improve the comparability of the results is to adjust the assay to match the results of the Hybritech Tandem™-PSA kit, which is widely used and established in the field. Correlation between assays does not necessarily mean that the same PSA subfraction is measured [1].

Recently it has been reported that immunoreactive PSA was present in 30% of breast tumor cytosols [3,4]. We attempted the development of a sandwich-type ELISA, employing monoclonal antibodies, to measure PSA with high sensitivity. The first aim has been a sensitivity which would allow the measurement of PSA in female sera.

Materials and Methods

A sandwich PSA ELISA was established by coating wells with the PSA mAB28A4 followed by two successive incubations with the sample and a successive incubation with PSA mAB43A2-peroxidase conjugate.

As PSA working standard we used concanavalin purified PSA from seminal fluid which was calibrated against highly purified PSA. As matrix we used the following solution: milk-powder (0.1%), BSA (2%),

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Tween (0.02%), thimerosal (0.1%), NaCl (0.3 mol/l), and Tris-HCl (0.04 mol/l, pH 7.4).

We have employed our PSA-ELISA to analyze n = 231 female sera with the tentative diagnosis of breast cancer according to increased concentrations of the tumor markers CA-15-3 and MCA. These sera had been selected from the laboratories of two of the authors.

Results

The lowest PSA concentration (µg/l) detectable by our PSA-ELISA was determined as 0.072 ± 2SD = 0.0865 (intra-test) or 0.0647 ± 2SD = 0.084 (inter-test). The precision of the assay was checked by eightfold analysis of seven different sera yielding a mean CV of 7.7%. PSA standards in the concentration range between 0.0626 to 4 µg/l were measured with both the Abbott IMx® PSA and the PSA-ELISA. For the PSA-ELISA linear regression furnished the relationship $y_1 = 0.019 + 0.991$ x (r= 0.997): for the Abbott IMx instrument data obtained were $y_2 = -0.098 + 0.547$ x (r > 0.999). Accordingly, the PSA-ELISA showed a higher slope factor indicating higher sensitivity.

In 31 of 231 (13.4 %) pathological sera we could detect PSA. The PSA concentrations have been classified into three categories: 0.104 - 0.195 µg/l (n = 19), 0.200 - 0.500 µg/l (n = 7) as well as 0.804 - 1.208 µg/l (n = 5).

Discussion

The prerequisite for the generation of sensitive immunoassay tests are monoclonal antibodies. It is evident that monoclonal antibodies are individual entities with the properties to recognize specific epitopes. It is highly probable that the PSA molecule carries multiple epitopes. Therefore it is a matter of chance only to generate monoclonal immunoglobulins with the properties to bind independently. It is mandatory to find out mABs against PSA that recognize major and sufficiently stabile epitopes. Obviously, these requirements are fulfilled by the monoclonal antibodies mAB43A2 and mAB28A4.

We found low but measurable PSA concentrations in human female sera. The significance of „PSA" in female sera is open for speculation.

As conceded by other authors [5], PSA can no longer be considered as an absolutely tissue-specific marker. Our results demonstrate that female sera cannot be regarded as PSA free sera. It may be advisable to generate a matrix to be used for PSA standards and zero standards.

Nonstandard abbreviations: CA-15-3, carcinoma-associated 15-3 antigen; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; mAB, monoclonal antibody; MCA, mucin-like carcinoma-associated antigen; PSA, prostate-specific antigen.

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