Biochip-based Test-system for Cancer Diagnostics. Simultaneous Quantitation of Total and Free Forms of Prostate-specific Antigen

T. Osipova*, Z.Sokolova*, T. Ryabykh*, V.Karaseva*, M.Modorsky**, V.Matveev*, A. Baryshnikov*

*N.N. Blokhin Cancer Research Center, RAMS, Kashirskiye shosse, 24, Moscow 115478, Russia, tatpav44@yandex.ru
** Hospital №60, Entuziastov shosse, 84/1, 111024 Moscow, Russia, gkb60@mosgorzdrav.ru

ABSTRACT

Diagnostic performance of biochip-based test-system for simultaneous quantitation of two forms of prostate-specific antigen (PSA) in human serum was evaluated and compared to efficacy of ELISA test-system (CanAg). Regression analysis revealed a high degree of correlation between the levels of total (PSAt) and free (PSAf) forms of PSA, measured by biochip-based system and CanAg system. ROC-analysis revealed that this new diagnostic system for simultaneous determination of PSAt, PSAf and of their ratio (%PSAf) can differentiate serum samples of the patients with prostate cancer (PCa) from those of healthy donors, patients with urogenital malignant tumors and benign prostate hyperplasia and has diagnostic efficiency highly competitive with that of traditional ELISA system. The advantage of biochip-based test system is simultaneous determination of both markers. This new test-system can be applied to PCa diagnostics in group of high risk (men older than 55).

Keywords: protein biochip, cancer diagnostics, PSAt, PSAf, ROC-analysis

1 INTRODUCTION

The application of nanotechnology to oncology can significantly improve diagnosis of cancer. Tumor seromarkers, such as tumor-associated antigens, are extensively used in modern clinical practice for cancer detection [1, 2]. Diagnostics using several tumor markers simultaneously is far more effective than in the case of one marker employment.

Biochip technology offers outstanding possibilities in the field of protein-based cancer diagnostics. This technology allows simultaneous determination of several (in the future – hundreds and thousand) biomarkers with the use of very small amounts of samples and reagents.

Microchips with three-dimensional gel elements (Engelhardt Institute of Molecular Biology (EIMB), RAS) having greater capacity compared with elements made by surface coating, exhibit more high sensitivity of protein testing [3, 4].

The first step on the way to elaboration of diagnostic system for simultaneous quantitation of several tumor markers was realizing of diagnostic system for analysis of two forms of prostate-specific antigen (PSA), prostate cancer (PCa) marker. This system is of interest to physicians by itself, as PCa remains the second leading cause of cancer death in men [5, 6]. PSA level is elevated in the blood of men with prostate cancer, so it is usable for detection of this cancer. The employment of two PSA forms – total (PSAt) and free (PSAf) and of their ratio (%PSAf) makes it possible to differentiate benign prostatic hyperplasia (BPH) from PCa and to reduce percent of unnecessary biopsies. Biochip technology opens up the new possibilities for these tumor markers applications to cancer screening.

It was demonstrated by us previously that new biochip-based test-system for determination of two PSA forms had a reasonable analytic performance assessed by evaluation of detection limit, within-run and between-day precisions and accuracy by studying the linearity of dilution and recovery rate [7].

In the clinical laboratory enzyme-linked immunosorbent assays (ELISA) set a golden standard for protein analysis. So new biochip-based test-system for cancer diagnostics needs verification by reference to ELISA system.

The goal of the investigation is diagnostic performance evaluation of the biochip-based test system for two PSA forms and its comparison to efficacy of ELISA test-system (CanAg).

2 MATERIALS AND METHODS

2.1 Structure of three-dimensional hydrogel-based microchips

The gel-based biochips (EIMB, RAS, Russia) were used in the investigation [8]. Such biochip represents an array of three-dimensional semi-spherical gel elements separated from each other with hydrophobic surface of glass slide. Each spot of the array represents individual micro container with volume of 0.1 nanoliter used for different reactions.
2.2 Participants and serum samples

The study included 171 patients with established diagnosis: 63 patients with PCa, 48 patients with benign prostate hyperplasia (BPH), 38 patients with urogenital malignant tumors (UMT) and 22 healthy donors, as well as 18 patients with unknown diagnosis. Three models were formed: PCa versus normal healthy controls, PCa versus UMT, and PCa versus BPH.

Blood samples were obtained: of cancer patients - from Department of Urology and Department of Clinical Immunology of N.N.Blokhin Cancer Research Center, RAMS; of healthy donors - from Hematological Research Center, RAMS, and of the patients with BPH - from Hospital #60, Moscow. The samples obtained were centrifuged at 1600g for 15 min. The sera were frozen and stored at -80°C until analysis.

2.3 Antigen, monoclonal antibodies and ELISA system

Purified antigen PSA was purchased from Xema-Medica, Moscow, Russia. Monoclonal antibodies against PSAt and PSAf were purchased from CanAg (Fujirebio Diagnostics), Sweden. Antibodies PSA30 recognizing PSA in a free form and antibodies PSA36, recognizing both total and free forms of PSA were used for on-chip immobilization. Antibodies PSA66 recognizing both total and free PSA forms were used as the second antibodies in sandwich immunoassay.

We used the enzyme immunometric assays (ELISA) CanAg PSAt and PSAf (Fujirebio Diagnostics, Sweden) as comparative methods. Data obtained by biochip-based system for simultaneous PSAt and PSAf determination were correlated with results obtained in CanAg systems. The CanAg PSA assay is based on the direct sandwich technique.

2.4 Biochip-based immunoassays

Antigen-capture one-site sandwich assay was used in the analysis. Microchips with immobilized by photo-induced co-polymerization monoclonal antibodies to PSAt and PSAf were used. They were treated with solutions of antigen or blood sera, and reaction was detected by the second antibodies labeled with fluorescent dye (Cy5). Fluorescent signals were recorded by a portable laser-based Fluorescence Biochip-analyzer (EIMB, RAS, Russia).

2.5 Analysis of data obtained and evaluation of diagnostic efficacy

For calculation of the PSAt and PSAf concentrations in human serum a standard curves were constructed by plotting fluorescence intensities against antigen concentrations in solution. The PSAt and PSAf concentrations in the unknown serum samples were determined from appropriate standard curves.

Statistical analysis.

Linear regression analysis was carried out with the statistical package Microsoft Excel 2003. The diagnostic test accuracy for both test-systems was evaluated by receiver-operating characteristics (ROC) curve analysis, mathematical analysis, which can evaluate sensitivity and specificity of diagnostic system [9]. The MedCalc statistical package for Windows, software version 9.3.5, was used for calculations.

3 RESULTS AND DISCUSSION

3.1 Determination of PSAt and PSAf levels in blood serum samples

New biochip-based test-system was examined with the use of clinical material, including sera of patients with PCa, BPH, UMT and of healthy donors.

It was found that the PSAt and PSAf levels, determined by standard curves in sera of healthy donors, did not exceed the cut-off levels well-known from the literature: 4 ng/ml and 1.1 ng/ml, respectively. The mean level of PSAt in sera of healthy donors (men) determined by biochip-based system represented 0.39 ng/ml and that of PSAf - 0.16 ng/ml.

Data obtained in biochip-based test-system for different groups of patients were compared with results derived in commercial CanAg test-system for the same serum samples. Regression analysis has revealed a high degree of correlation for data obtained in both systems. Correlation coefficients of the results realized in two systems both for PSAt and for PSAf concentrations in the serum samples were 0.96 (p<0.0001) and 0.962 (p<0.0001), respectively. Fig. 1 represents lines of regression for the levels of PSAt and PSAf realized in both systems.

Thus biochip-based system makes possible simultaneous quantitation of both PSA forms and realizing results correlated with data obtained in traditional ELISA system.

3.2 ROC analysis and diagnostic validity

ROC-analysis was performed for evaluation of diagnostic efficacy of biochip-based test-system for determination of two PSA forms and for its reference to diagnostic efficacy of standard ELISA system (CanAg).

We evaluate ability of the new system to differentiate: 1) the group of patients with PCa from the group of healthy donors; 2) the group of patients with PCa from the group of patients with UMT and 3) the group of patients with PCa from the group of patients with BPH.

Fig. 2 represents examples of ROC-curves of PSAt (a)
and PSAf (b) for the model PCa versus healthy donors. The area under the curves (AUC) is 0.747 for PSAt and 0.679 for PSAf, what indicates that biochip-based test-system has reasonable diagnostic efficacy.

ROC-analysis permits not only to evaluate diagnostic efficacy of a system, but also to compare diagnostic efficacies for two or more systems by use AUCs. Fig 3 illustrates two ROC-curves for %PSAf resulting from sera analysis by biochip and by CanAg systems for the model PCa versus BPH. As is seen from Fig.3, AUCs for the both test-systems are closely related to each other, differences are not statistically significant (p>0.05).

Table 1 summarizes data obtained when compare two diagnostic systems using all the models involved. As will be seen from the table, diagnostic efficacy of biochip-based test-system compares well with that of the standard ELISA system, CanAg. Differences in AUCs are not statistically

Fig. 2. ROC-curves of PSAt (a) and PSAf (b). The model PCa versus healthy donors

Fig. 3. A comparison of ROC-curves of %PSAf obtained with biochip-based test-system (continuous line) and CanAg system (broken line). The model PCa versus BPH
Table 1. Area under the ROC-curves for PSA\textsubscript{t}, PSA\textsubscript{f} and %PSA\textsubscript{f} obtained with biochip-based test-system and CanAg system for all the models involved

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Variable</th>
<th>Test-system</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>microchip</td>
<td>CanAg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AUC</td>
<td>AUC</td>
</tr>
<tr>
<td>PCa / Healthy donors</td>
<td>85</td>
<td>PSA\textsubscript{t}</td>
<td>0.747</td>
<td>0.768</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSA\textsubscript{f}</td>
<td>0.679</td>
<td>0.713</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%PSA\textsubscript{f}</td>
<td>0.961</td>
<td>0.915</td>
</tr>
<tr>
<td>PA / UMP</td>
<td>101</td>
<td>PSA\textsubscript{t}</td>
<td>0.809</td>
<td>0.836</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSA\textsubscript{f}</td>
<td>0.713</td>
<td>0.766</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%PSA\textsubscript{f}</td>
<td>0.701</td>
<td>0.599</td>
</tr>
<tr>
<td>PCa / BPH</td>
<td>111</td>
<td>PSA\textsubscript{t}</td>
<td>0.556</td>
<td>0.504</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSA\textsubscript{f}</td>
<td>0.495</td>
<td>0.516</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%PSA\textsubscript{f}</td>
<td>0.725</td>
<td>0.629</td>
</tr>
</tbody>
</table>

significant in all cases.

Thus microchip-based diagnostic system for simultaneous quantitation of PSA\textsubscript{t}, PSA\textsubscript{f} and of theirs ratio (%PSA\textsubscript{f}) can differentiate serum samples of the patients with PCa from these of healthy donors, patients with UMP and BPH. A comparison between diagnostic performance of biochip-based system and traditional diagnostic system CanAg revealed that new test-system has diagnostic efficiency highly competitive with that of traditional ELISA system.

The advantage of biochip-based test system is simultaneous determination of both markers. This new test-system can be applied to PCa diagnostics in group of high risk (men older than 55).

REFERENCES