Correlation of high-molecular cytokeratin in tissue of prostatic cancer with Gleason score and PSA

The absence of basal cell layer of prostatic acini containing high-molecular cytokeratin, which is immunohistochemically detected by monoclonal antibody 34βE12, is an essential diagnostic characteristic of prostatic cancer. The absence of immunohistochemical reaction in 3 or more pseudoglandular structures of prostatic tissue indicates malignant process. The percentage of immunohistochemically completely negative glandular structures was determined by semiquantitative measurement in tissue specimens obtained by TRUS biopsy of the prostate, and it was correlated with serum PSA concentration and Gleason score. The increase of percentage of glandular prostatic formations completely negative to high-molecular cytokeratin detected by 34βE12 led to simultaneous rise of mean value of Gleason prostatic cancer score (p<0.001) as well as the average serum PSA concentration in subjects (p<0.05).

Key words: prostatic cancer, basal cells, high-molecular cytokeratin, PSA, Gleason score

INTRODUCTION

Cancer of prostate is the most frequent form of visceral cancers in men and one of the leading causes of death; however, diagnostic and prognostic criteria have not been defined yet. Diagnosis of prostatic cancer is based on evaluation of histological and cytological changes, and recently high significance has been attributed to immunohistochemical analysis. Histological structural changes are reflected in irregular arrangement of pseudoglandular formations, while cytological characteristics include the enlarged cell and nuclei with prominent nucleoli which are large, eccentric and multiple in cancer cells.

The loss of basal cell layer of prostatic acini is one of the essential characteristics of prostatic adenocarcinoma.\(^{1,2}\) Such finding discovered by light microscopy, was first highlighted by Lewis in 1950, and in 1953, it was elaborated as diagnostic criterion by Totten and associates.\(^{1,2}\) With the advent of immunohistochemistry, the specific staining to cytokeratin in basal cell layer of prostatic acini has become a beneficial supplemental to diagnosis of adenocarcinoma. Cytokeratins belong to the family of hydrophilic proteins, they form the cytoskeleton in epithelial cells and represent one of the most significant tumor markers of prostate. Introduction of monoclonal antibody 34βE12, binding to cytokeratin filaments of large molecular mass (53-67 kDa), present in basal and absent in secretory cells of prostatic acini, enabled clear visualization of basal cells of benign prostatic acini, which are otherwise absent in malignant glandular structures of prostatic cancer.\(^3\) In this procedure, secretory and stromal cells remain immunohistochemically unstained.

The objective of this study was to establish the correlation of percentage prevalence of immunohistochemical negative glandular structures to cytokeratin with other diagnostic and prognostic prostatic cancer parameters, such as serum prostatic specific antigen (PSA) concentration in patients\(^4\) and Gleason score.\(^4\)

MATERIAL AND METHODS

Tissue specimens of puncture biopsies obtained by True Cut needle were analyzed, on what basis the diagnosis of prostatic cancer was made in 40 patients. Transrectal ultrasound guided (TRUS) target and randomized biopsies (10-12 specimens) of prostate were performed at the Institute of Urology and Nephrology, Clinical Center of Serbia, in the period 2000 to 2005. If the volume, determined by transrectal ultrasound technique, was up to 45 ml, 10 specimens were excised, and when it was over 45 ml, our option was 12 specimens. Serum concentrations of prostatic specific antigen were measured for every patient as well as the ratio of free and total (f/T) PSA in serum before the digital rectal examination and TRUS biopsy.
Tissue specimens, upon formalin fixation and paraffin molding, were stained by hematoxylin and eosin and examined by optic microscopy. The sections containing atypical or malignant acini were immunohistochemically stained by monoclonal antibody 34βE12 produced by DAKOPATS (AE1/AE3 clones, IgG class, kappa subclass). Paraffin sections were treated by standard peroxidase streptavidin technique DAKO LSAB+. Labeling system made of solution containing streptavidin conjugated with peroxidase was used for visualization. The procedure was completed by tissue incubation with chromogenic diaminobenzidine (DAB) substrate, which is noted as brown precipitate at the locus of antigen. Tissue specimens were contrasted by Mayer's hematoxylin.

Upon verification of the absent high-molecular cytokeratin, the cancer was histologically graded and scored by Gleason scale. Finally, percentage prevalence of acini completely unstained by antibody 34βE12 was semiquantitatively computed in these tissue specimens, and distributed in categories illustrated in Table 1. Unifactorial analysis of variance (ANOVA) was used for statistical testing of results.

RESULTS

In the analyzed group of 40 patients with prostatic cancer, Gleason scores were in the interval from 4 to 9. Score 4 was found in 11 patients, score 5 in 5 cases, and score 6 in 14 patients, being the most prevalent. Score 7 was measured in 5 patients, score 8 in 3, and score 9 in 2 patients.

Immunomorphological analysis of the expression of high-molecular cytokeratin showed that the largest number of patients (22) belonged to the group II (Table 1), in which 30% to 70% of glandular structures of biotic specimens were without basal cells. Among them, five patients were scored 4 by Gleason scale, four were scored 5, and nine were scored 6. Score 7 was measured in three patients, while score 8 was found in one patient.

Thirteen patients belonged to the group where more than 70% of glandular structures of biotic specimens were malignantly transformed, with completely absent basal cell layer. Within this group, there were several subgroups divided by Gleason score, i.e. one patient was with scores 4 and 5, respectively, and 5 patients with score 6, and 2 patients with scores 7.8 and 9, respectively.

Only five patients had less than 30% of completely unstained glandular structures. In this group, all five patients were scored 4.

Serum PSA level ranged from 3.0 ng/ml to 150 ng/ml, and F/T PSA ratio was 0.05 to 0.8. (Table 1)

Percentage of glandular prostatic formation completely negative to high-molecular cytokeratin (antibody 34E12)

Table 1 shows that higher percentage of glandular prostatic formations completely negative to high-molecular cytokeratin detected by antibody 34βE12 simultaneously leads to rise of Gleason score of prostatic cancer (p<0.001) and serum PSA concentrations (p<0.05).

DISCUSSION

The demand for timely detection of prostatic cancer has been increasing, because it allows for better outcome of the disease. Screening by PSA, F/T PSA determination and rectal examination of the prostate enables the selection of patients for biopsy and early pathohistological diagnosis of the condition. Accordingly, the patients with
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ADENOCARCINOMA OF PROSTATE GLEASON 3, EXPRESSION OF HIGH-MOLECULAR CYTOKERATIN 34BE12, 20X MAGNIFICATION. THE ABSENCE OF EXPRESSION IN GLANDS WITH DEFICIT OF BASAL LAYER IS NOTED.

FIGURE 3.

ADENOCARCINOMA OF PROSTATE GLEASON 3, EXPRESSION OF HIGH-MOLECULAR CYTOKERATIN 34BE12, MAGNIFICATION 20X. THE ABSENCE OF EXPRESSION IN GLANDS WITH DEFICIT OF BASAL LAYER IS NOTED.

FIGURE 4.

Predominantly low PSA and F/T PSA values were analyzed in this study, what enabled early pathohistological diagnosis of prostatic cancer. Therefore, the majority of our patients had prostatic cancer of low Gleason’s score. As it was previously described, immunohistochemical analysis of high-molecular cytokeratin has a significant supporting place in delicate cases, for better spotting of basal cells that are undetectable in malignant glandular structures.

The essential diagnostic value of monoclonal antibody 34BE12 is differentiation of benign lesions, such as atypical glandular foci, adenosis, prostatic intraepithelial neoplasia, basal cell hyperplasia and atrophy of prostatic acini, from the malignant glandular structure of similar appearance. Sometimes, however, discontinuous staining of basal cell layer may be observed in apparently benign glandular structures of the prostate.

On the other hand, there is no minimal number of glandular prostatic acini with negative staining to antibody 34BE12 below which the diagnosis of prostatic cancer is impossible (cut off), i.e. just one immunohistochemically negative acinus is quite enough for diagnosis. However, if there is three or more pseudoglandular formations with completely negative staining to this antibody, then the diagnosis of prostatic cancer may be made with certainty, along with other morphological criteria. Nevertheless, it was noted that, in less than 1.1% of cases, focal positivity to high-molecular cytokeratin reacting with 34BE12 antibody, might be found in apparently malignant prostatic acini, pertaining to rare basal cells. Diagnostic criteria, in these cases, were the signs of nuclear atypical feature - enlarged and hyperchromatic nuclei and marked nucleoli. It is necessary to emphasize that, in biopsy of prostate, the most frequent differential-diagnostic problem is the finding of small atypical glandular structures, which will be recognized as benign in case of positive immunohistochemical staining, and as malignant in the event of negative staining, with Gleason score 6.

Considering the described diagnostic overlapping in the application of monoclonal antibody 34BE12 as well as the deficiency of diagnostic cut off values for the number of negative staining of acinar structures, it was intriguing to review the validity of this marker of basal cells through the correlation of diagnostically relevant clinical parameter such as serum PSA concentration and prognostically worthy morphological indicator such as Gleason score.

For prostatic cancer, I category prognostic markers are those whose worth and utility in clinical practice have been unquestionably proved. These are preoperative serum PSA concentration and Gleason score. Both parameters are significant for opting for therapeutic procedure of prostatic cancer.

Ratio of free and total serum PSA concentration is in the III category of predictive parameters whose verification requires broader research, what is in the line with described results in this text.

According to results of some authors, the percentage of involvement of puncture biopsy specimen by prostatic cancer tissue (median 47%) is not significantly correlated with survival of patients, but serum PSA concentration and Gleason score are significant. On the basis of presented results in this study, it may be stated that there is an indirect correlation with patients’ survival and it is exhibited, before all, by highly significant correlation with Gleason score. Such statement is corroborated by very significant correlation of Gleason score and PSA (p<0.001).

Our study, however, analyzed the percentage prevalence of immunohistochemically detected glandular formations of the prostate without basal cell layer in the puncture biopsy specimen, what solved all aforementioned dilemmas present in the regular optic microscopic diagnosis of the prostatic cancer and evaded all possible overlapping in both directions.
CONCLUSION

Our study has confirmed that monoclonal antibody 34βE12, besides its verified role in objectivity of histopathological diagnosis of prostatic cancer, has indirect prognostic value, owing to significant correlation with mean PSA values and Gleason scores.

REZIME

Nedostatak bazalnog sloja ćelija acinus prostate koje sadrže visokomolekularni citokeratin, a koji se immunohistohemijski detektuje monoklonskim antitelom 34βE12, bitno je dijagnostičko svojstvo karcinoma prostate. Odsustvo immunohistohemije reakcije u 3 ili više pseudoglandularnih struktura u tkivu prostate ukazuje na posto-

jaje malignog procesa. Semikvanitativno je određen procenat immunohistohemija potpuno negativnih glandularnih struktura u tkivnim uzorcima dobijenim TRUS biopsijom prostate i analizirana korelacija sa koncentracijom PSA u serumu bolesnika i Glišnovim skorom. Sa porastom procenta pseudoglandularnih formacija prostate potpuno negativnih na visokomolekularni citokeratin detekovan antitelim 34βE12 raste srednja vrednost Glišnovog skora karcinoma prostate (p<0.001), kao i prosečna koncentracija prostatičnog specifičnog antigena (PSA) u serumu ispitanika (p<0.05).

Ključne reči: karcinom prostate, bazalne ćelije, visokomolekularni citokeratin, PSA, Glišnov skor
TABLE I

MEAN VALUES (±SD) OF GLEASON SCORE OF PROSTATIC CANCER AND SERUM PSA CONCENTRATIONS OF PATIENTS IN RELATION TO THE PERCENTAGE OF COMPLETELY NEGATIVE PROSTATIC ACINI TO HIGH-MOLECULAR CYTOKERATIN (ANTIBODY 34βE12)

<table>
<thead>
<tr>
<th>Percentage of glandular prostatic formation completely negative to high-molecular cytokeratin (antibody 34βE12)</th>
<th>No of pts</th>
<th>Gleason score</th>
<th>Serum PSA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30%</td>
<td>5</td>
<td>4.00+/-.00</td>
<td>7.743.30</td>
</tr>
<tr>
<td>30-70</td>
<td>22</td>
<td>5.591.14</td>
<td>12.005.88</td>
</tr>
<tr>
<td>&gt;70%</td>
<td>13</td>
<td>6.691.49</td>
<td>36.7244.59</td>
</tr>
<tr>
<td>ANOVA (unifactorial signifcance)</td>
<td></td>
<td>F=9376,p&lt;0.001</td>
<td>F=4.344,p=0.020</td>
</tr>
</tbody>
</table>

GRAFIKON
SREDNJE VREDNOSTI (± SD) KONCENTRACIJE PSA U SERUMU U ZAVISNOSTI OD GLISONOVOG SKORA KARCINOMA PROSTATE

REFERENCES