Gostota malih žil in vrednosti PSA pri adenokarzinomih prostate z oceno 6 in 7 po Gleasonu
Microvessel density and PSA level in prostatic adenocarcinoma of Gleason score 6 and 7

**Abstract**

**Purpose:** The aim of this investigation was to determine the differences in microvessel density (MVD) and serum levels of prostate-specific antigen (PSA) between groups of patients with Gleason score (GS) 6 prostatic adenocarcinoma and patients with GS 7 prostatic adenocarcinoma.

**Methods:** The study included a series of 100 patients with prostatic adenocarcinoma. Tumor specimens were divided into two groups: GS 6 (52 cases) and GS 7 (48 cases). Intratumoral microvasculature was determined by immunohistochemistry using an antibody against endoglin. Endoglin stained microvessels were observed in and around the tumor, but weak or no staining of blood vessels was seen in non-neoplastic tissue. Areas of maximal angiogenesis within the tumor were identified and microvessels were counted at ×400 magnification (0.19 mm² field).

**Results:** The GS 6 specimens did not significantly differ in MVD per field (24.5 vs. 29.0; P = 0.46) or in PSA values. A significant difference in PSA levels was observed between groups: 6.6 vs. 10.4 ng/ml (P = 0.0005).

**Conclusion:** The results suggest that patients with GS 6 prostate adenocarcinoma have a lower MVD and lower PSA levels compared to patients with GS 7 prostate adenocarcinoma.

**Key words:** prostatic adenocarcinoma, angiogenesis, microvessel density, endoglin, immunohistochemistry.
INTRODUCTION

Prostatic adenocarcinoma is the most commonly diagnosed male malignancy and its incidence is growing (1, 2). Parameters that can stratify patients for type of therapy based on the likelihood of tumor progression are clinical stage, serum level of prostate-specific antigen (PSA) and histological differentiation, which is conventionally reported as the Gleason score (3, 4). Approximately 80% of men diagnosed with prostate cancer have moderately increased serum levels of PSA (3–10 ng/mL) and a non-palpable localized tumor with a Gleason score of 6 or 7 (GS 6 or 7) (5, 6). However, elevated serum PSA levels can be detected in non-tumor disease, including benign prostate hyperplasia and prostatitis (1). Furthermore, for patients with an intermediate GS (GS 6 and GS 7), accurate predictions of outcome are often difficult (1). Therefore, many investigators pay special attention to tumor markers and predictive factors in patients with prostatic adenocarcinoma.

Angiogenesis is the formation of new blood vessels from pre-existing vessels and has an important role in the progression and metastasis of tumors (7). The most common method for semi-quantitative evaluation of angiogenesis is the measurement of microvessel density (MVD) using endothelial markers (8). Endoglin (CD 105) is a transforming growth factor 1 receptor. It is expressed on endothelial cells during tumor angiogenesis and inflammation with weak or negative expression in the vascular endothelium of normal tissue (8-11). MVD evaluation determined using anti-endoglin monoclonal antibodies has been shown to be an independent prognostic factor for certain types of malignant neoplasia, such as breast carcinoma and non-small-cell lung carcinoma (12, 13). Nevertheless, its significance in prostatic adenocarcinoma is controversial, as some studies proved a correlation between MVD and both tumor progression and survival (9, 14-18), while others failed to confirm the prognostic value of MVD (1, 19-21).

The aim of the present study was to determine possible differences in MVD (assessed by analyses of endoglin immunoreactivity) and serum levels of PSA between groups of patients with GS 6 prostatic adenocarcinoma and patients with GS 7 prostatic adenocarcinoma. Here we present the final results of the first study of endoglin expression in prostatic adenocarcinoma in subjects living in Slovenia.

MATERIALS AND METHODS

The 105 radical prostatectomy tissue specimens were re-examined. Of these, 100 were considered suitable for the study (paraffin blocks intact, enough material for re-cutting, complete baseline clinical and follow-up data). The median age of the patients at diagnosis was 65 (range 44–74) years. The specimens were divided in two groups: GS 6 (52 cases) and GS 7 (48 cases). Paraffin-embedded biopsy tissue blocks were cut into 4-µm sections, deparaffinized, and rehydrated.
Antigenic recovery was achieved by heating the slides in an autoclave with sodium citrate buffer (30 min). Endogenous peroxidase was inhibited with a Peroxidase Block Kit (Novocastra Laboratories, Newcastle upon Tyne, UK). Immunohistochemical staining was undertaken using primary antibodies against endoglin (1:50 dilution; Novocastra Laboratories). A Novolink Polymer Detection System (Novocastra Laboratories) was used for visualization. Primary antibodies were omitted in negative controls. Sections of tonsil tissue were used as positive controls. Tissue sections were counterstained using Mayer’s hematoxylin and mounted. Immunoreactivity was evaluated without knowledge of patient data. After scanning the immunostained section at low magnification (×40), three areas of maximal angiogenesis (“hotspots”) within the tumor were identified. Microvessels were then counted at ×400 magnification (0.19 mm² field). Any single cell or spot stained by the immunohistochemical marker was counted as a vessel. As in previous reports (9, 12, 17), a visible vascular lumen was not required to count as a microvessel. The highest number of vessels counted in any hotspot was recorded (MVD per field). The mean vascular count per mm² was then calculated (MVD per mm²). Both values were used in the statistical analysis. The groups were compared using the Student’s t-test for independent samples. Correlations were calculated using Pearson’s correlation test. P<0.05 was considered significant. Statistical analyses were carried out using IBM SPSS (Version 25.0. IBM Corp., Armonk, NY, USA).

RESULTS

The group of specimens with GS 6 had lower MVD per field than the group with GS 7 (24.5 vs. 29.0; P=0.46; Table 1), but this difference was not significant. The same was true when MVD per mm² was compared between the two groups (109.3 vs. 129.6; P=0.78; Table 1). Endoglin expression in GS 6 and GS 7 specimens is shown in Figures 1 and 2, respectively. The preoperative serum level of PSA was 1.4–69.5 ng/mL in the GS 6 group (median, 5.7 ng/mL), and 0.3–34.4 ng/mL in the GS 7 group (median, 8.1 ng/mL; Figure 3). The mean PSA level in serum was not significantly different in the GS 6 group compared with the GS 7 group (8.5 vs. 10.1 ng/mL; P=0.66; Table 1). MVD per mm² was not correlated with PSA (r=0.1; P=0.62). The age of patients at diagnosis was not significantly different in the two groups (63.0 vs. 65.0 years; P=0.84; Table 1).

DISCUSSION

In this study, we investigated angiogenesis in GS 6 and GS 7 specimens, currently the most commonly assigned Gleason scores in prostatic adenocarcinomas (4). Angiogenesis plays an important role in tumor growth and cancer cell dissemination. The association between increasing tumor vascularity and various measures of tumor aggressiveness (such as a greater incidence of metastases and/or reduced patient survival) has been shown in studies of patients with various types of carcinoma (12, 13, 15, 22-24). Our results showed no significant difference in MVD in GS 6 specimens compared with GS 7 specimens. Some studies have shown a correlation between Gleason score and MVD (9, 16-19, 25), whereas others have not (1, 20, 26–28). This discrepancy may have been due to the different methodology used to assay MVD in the above-mentioned studies. It has been reported that the choice of antibody (e.g., CD31, CD34, von Willebrand factor (vWF), endoglin) can influence study outcome (29). Although CD31, CD34, and vWF do not stain all microvessels, and particularly not newly formed microvessels (9, 17, 25),

Table 1: Comparison of GS 6 and GS 7 specimens

<table>
<thead>
<tr>
<th></th>
<th>GS 6 (n=52)</th>
<th>GS 7 (n=48)</th>
<th>P value</th>
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<tbody>
<tr>
<td>MVD per field</td>
<td>24.5 ± 13.5</td>
<td>29.0 ± 13.9</td>
<td>0.46</td>
</tr>
<tr>
<td>MVD per mm²</td>
<td>109.3 ± 58.2</td>
<td>129.6 ± 64.7</td>
<td>0.78</td>
</tr>
<tr>
<td>PSA (ng/mL)</td>
<td>8.5 ± 11.1</td>
<td>10.1 ± 8.2</td>
<td>0.66</td>
</tr>
<tr>
<td>Age of patients</td>
<td>63.0 ± 5.3</td>
<td>65.0 ± 6.4</td>
<td>0.84</td>
</tr>
</tbody>
</table>

GS = Gleason score; SD = standard deviation; MVD = microvessel density; PSA = prostate-specific antigen

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several authors used these antibodies (14, 16, 18, 19, 26, 28). In the present study, we used endoglin, which was consistently present in all cases and which stained microvessels in and around the tumor, but showed weak or no staining of blood vessels in non-neoplastic tissue. Studies also differed with regard to the quantification of angiogenesis, as most authors examined areas of maximal angiogenesis (hotspots) at ×200 magnification (6, 9, 16, 18, 19, 25). In the present study, we evaluated angiogenesis at ×400 magnification, which allowed more precise quantification of the number of vessels than if we had evaluated MVD at ×200 magnification. Only a few reports determined MVD at ×400 magnification (14, 17, 28). Furthermore, in the statistical analyses we used two series of data for each specimen: MVD per field and MVD per mm². Nevertheless, according to our results GS 6 and GS 7 specimens do not differ in the angiogenic status of cancer tissue. Two recent research studies also failed to prove MVD as a prognostic factor in prostatic adenocarcinoma (1, 20). No correlation between MVD and serum levels of PSA was observed in the present study. This finding is in agreement with those in other reports (19, 25, 27, 28). Furthermore, a significant difference was not shown when serum levels of PSA between the two groups of patients in the present study were compared. One reason for this is the degree of dispersion of the data. PSA is a key variable in prognostic models for clinically localized prostate cancer. It is used to assess pathological tumor stage and the risk of disease recurrence after local therapy. However, elevations in
PSA serum levels do not solely reflect the presence of cancer, but may also be driven by certain non-malignant causes such as nodular hyperplastic changes in the prostate gland, and prostatic inflammatory processes (30). In fact, PSA is not a cancer-specific but an organ-specific marker (31). Several studies have reported the limitations and inconsistency of PSA as a diagnostic and prognostic marker for prostate cancer (25-28, 30-33). Consequently, other PSA-based strategies are being tested for clinical use, such as PSA density (ratio of an individual serum PSA level and its corresponding prostate volume as assessed by transrectal ultrasonography), percent free PSA (%fPSA; calculated from analyzed free PSA and total PSA) and complexed PSA (bound to plasma proteins). Of these, only %fPSA is already used in the clinic (30). Furthermore, several alternative biomarkers of the cell cycle, cell invasion, cell adhesion, signal transduction, apoptosis, angiogenesis, and genetic biomarkers have been suggested to supplement or even replace PSA to improve strategies for early detection and predict the natural behavior of the tumor (31, 33).

In summary, our results showed that GS 6 and GS 7 prostate tumors do not significantly differ in MVD and serum levels of PSA. Although MVD has already been found to be a prognostic factor in several malignancies, this is still controversial in prostatic adenocarcinoma. One of the reasons for this is that a standard method with high reliability and reproducibility has not yet been established. Furthermore, new information regarding markers and antibodies for cancer-specific endothelial cells has increased in recent years. Taken together, these findings suggest that more detailed studies with large numbers of specimens and a precise methodology for evaluating angiogenesis are needed to understand the pathological role of angiogenesis in prostatic adenocarcinoma and elucidate the possible prognostic value of MVD.

REFERENCES


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