

Primerjava gostote malih žil in vrednosti PSA pri adenokarcinomih prostate z oceno 6 in 7 po Gleasonu

Comparison of microvessel density and PSA level in prostatic adenocarcinoma of Gleason score 6 and 7

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Ključne besede:

adenokarcinom prostate, angiogeneza, endoglin, gostota malih žil, imunohistokemija.

Key words:

prostatic adenocarcinoma, angiogenesis, endoglin, microvessel density, immunohistochemistry.

Članek prispel / Received

27.02.2012

Članek sprejet / Accepted

19.03.2012

Naslov za dopisovanje /

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Izvleček

Namen: Želeli smo ugotoviti, ali se bolniki z adenokarcinomom prostate z oceno 6 in 7 po Gleasonu razlikujejo v gostoti malih žil v tumorjih in vrednostih prostatičnega specifičnega antigena (PSA).

Metode: V raziskavo je bilo vključenih 26 bolnikov z adenokarcinomom prostate, ki smo jih razdelili v dve skupini glede na oceno po Gleasonu (GS). V prvi skupini je bilo 13 bolnikov z GS 6 in v drugi 13 bolnikov z GS 7. S protitelesi za endoglin smo raziskali ožiljenost znotraj tumorja. Endoglin je specifičen označevalec za novo nastale žile v in okrog tumorskega tkiva, ne pa za žile v netumorskem tkivu. Na vsakem histološkem preparatu smo določili območja največje gostote malih žil in

Abstract

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

Methods: The study was done in a series of 26 patients with prostatic adenocarcinoma. Specimens were divided into two groups: GS 6 (13 cases) and GS 7 (13 cases). Intratumoral microvasculature was highlighted by immunohistochemical means using an antibody against endoglin. Endoglin stained microvessels in and around the tumor but showed weak or no staining for blood

le-te prešteli pri 400-kratni povečavi (površina 0,19 mm²). **Rezultati:** Skupina vzorcev z GS 6 je imela manjšo gostoto žil v vidnem polju (19,9 vs. 25,6; $P = 0,05$) in manjšo gostoto žil, preračunano na mm² tumorskega tkiva (89,7 vs. 117,7; $P = 0,05$) v primerjavi s skupino vzorcev z GS 7. Skupini se nista razlikovali glede na vrednost PSA (5,7 vs. 10,2 ng/mL; $P = 0,10$). Gostota malih žil v tumorjih ni bila povezana z vrednostjo PSA ($r = 0,20$; $P = 0,30$). Bolniki z GS 6 so bili mlajši od bolnikov z GS 7 (61,2 vs. 66,2 let; $P = 0,01$).

Zaključek: Predhodni rezultati raziskave kažejo na verjetnost, da se adenokarcinomi prostate z oceno 6 in 7 po Gleasonu razlikujejo v neovaskularizaciji, ne pa tudi v vrednostih PSA, kar nameravamo potrditi s prihodnjo študijo večjega števila primerov.

vessels in non-neoplastic tissue. Areas of maximal angiogenesis within the tumor were identified and microvessels counted at $\times 400$ magnification (0.19 mm² field).

Results: The GS 6 group had lower MVD per field (19.9 vs. 25.6; $P=0.05$) and MVD per mm² (89.7 vs. 117.7; $P=0.05$) when compared with the GS 7 group. No significant difference in mean serum levels of PSA between the two groups was observed (5.7 vs. 10.2 ng/mL; $P=0.10$). MVD per mm² was not correlated with PSA ($r=0.20$; $P=0.30$). The age of patients at diagnosis was significantly lower in cases with GS 6 prostatic adenocarcinoma (61.2 vs. 66.2 years; $P=0.01$).

Conclusion: Our preliminary results suggest a possible difference in the neovascularization between the two groups of specimens, but a difference in PSA level was not proved. Nevertheless, a future large-scale study is needed to confirm these findings.

INTRODUCTION

Angiogenesis is the formation of new blood vessels. Capillaries "sprout" from pre-existing vessels and have an important role in the progression and metastasis of tumors (1, 2). Studies have suggested that the microvessel density (MVD) of prostatic adenocarcinoma may be of prognostic value (3–7). Endoglin (CD 105) is a receptor for transforming growth factor $\beta 1$. It is expressed on endothelial cells during tumor angiogenesis and inflammation with weak or negative expression in the vascular endothelium of normal tissue (5, 8–12). MVD evaluation as 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 (13, 14).

Adenocarcinoma of the prostate gland is the most commonly diagnosed male malignancy in the Euro-

pean Union and USA (15, 16). Parameters that can stratify patients for type of therapy based on likelihood of tumor progression are clinical stage, serum levels of prostate-specific antigen (PSA) and histological differentiation, which is conventionally reported as the Gleason score (17, 18). 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) (19, 20). For prostatic adenocarcinoma with a GS 6, the clinical course is often unpredictable. Hence, MVD as a possible independent prognostic factor could be of great value.

A study of endoglin expression in prostatic adenocarcinoma in subjects living in Slovenia has not been carried out. The aim of our investigation was to determine possible differences in MVD (assessed by analy-

ses of endoglin immunoreactivity) and serum levels of PSA between groups of patients with GS 6 adenocarcinoma and patients with GS 7 adenocarcinoma. We report here the preliminary results of the study.

MATERIALS AND METHODS

Thirty tissue specimens of radical prostatectomy were re-examined. Twenty-six were considered suitable for the study (paraffin blocks intact, enough material for re-cutting, basal clinical and follow-up data complete). The median age of patients at diagnosis was 64 (range 53–71) years. Specimens were divided in two groups: GS 6 (13 cases) and GS 7 (13 cases). All cases were stage pT2. Paraffin-embedded biopsy tissue blocks were cut into 4- μ m 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. As positive controls, sections of tonsil tissue were used. 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 ($\times 40$), three areas of maximal angiogenesis ("hotspots") within the tumor were identified. Then, microvessels were counted at

$\times 400$ magnification (0.19 mm² field). Any single cell or spot stained by the immunohistochemical marker was counted as a vessel. As in previous reports (5, 7, 13), 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). Then the mean vascular count per mm² was calculated (MVD per mm²). Both values were used in the statistical analysis. Groups were compared with Student's t-test for independent samples. Correlations of MVD and preoperative PSA level were calculated using Pearson's correlation test. $P < 0.05$ was considered significant. Statistical analyses were carried out using SPSS ver19 (SPSS, Chicago, IL, USA).

RESULTS

Endoglin expression in specimens with GS 6 and GS 7 is shown in Figures 1 and 2, respectively. The group of specimens with GS 6 had lower MVD per field than the group with GS 7 (19.9 vs. 25.6; $P = 0.05$; Table 1), but this difference was not significant. The same was true when MVD per mm² was compared between the two groups (89.7 vs. 117.7; $P = 0.05$; Table 1). The preoperative serum level of PSA was 1.8–8.8 ng/mL in the GS 6 group (median, 5.6 ng/mL), and 2.8–34.4 ng/mL in the GS 7 group (median, 5.9 ng/mL; Figure 3). The mean PSA level in serum was not significantly different in GS 6 group compared with the GS 7 group (5.7 vs. 10.2 ng/mL; $P = 0.10$; Table 1). MVD per mm² was not correlated with PSA ($r = 0.20$; $P = 0.30$; Fig 4). The age of patients at diagnosis was significantly lower in cases with GS 6 prostatic adenocarcinoma (61.2 vs. 66.2 years; $P = 0.01$; Table 1).

Table 1: Comparison of GS 6 and GS 7 specimens

| | GS 6 (n=13) Mean \pm SD | GS 7 (n=13) Mean \pm SD | P value |
|-------------------------|-------------------------------------|-------------------------------------|----------------|
| MVD per field | 19,9 \pm 7,2 | 25,6 \pm 7,2 | 0,05 |
| MVD per mm ² | 89,7 \pm 32,2 | 117,7 \pm 38,7 | 0,05 |
| PSA (ng/mL) | 5,7 \pm 2,2 | 10,2 \pm 9,1 | 0,10 |
| Age of patients (years) | 61,2 \pm 4,7 | 66,2 \pm 3,9 | 0,01 |

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

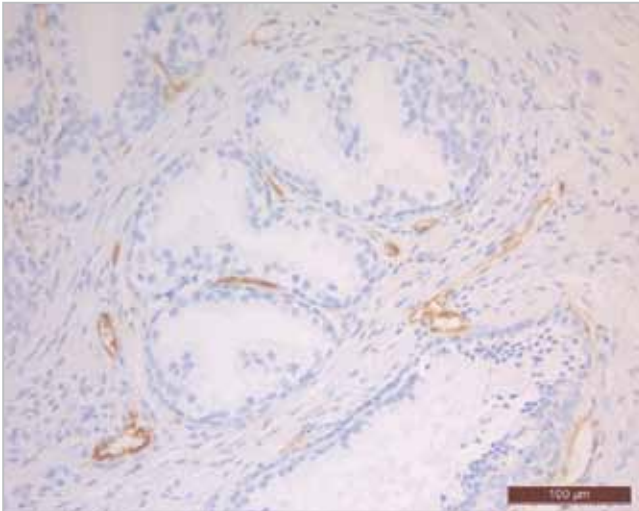


Figure 1. Immunohistochemical endoglin expression in GS 6 specimen

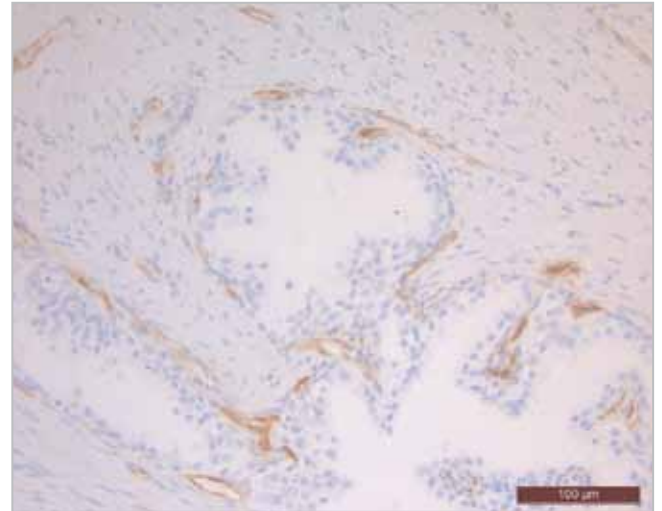


Figure 2. Immunohistochemical endoglin expression in GS 7 specimen

DISCUSSION

Angiogenesis enables tumor growth and metastasis by providing nutrients and oxygen for metabolism as well as removal of the resultant waste products. Angiogenesis initially develops by incorporating existing blood vessels, but solid tumors cannot probably grow $>1 \text{ mm}^3$ unless they synthesize their own network of morphologically and functionally primitive and abnormal microvessels (i.e., neovascularization) (14, 21–

23). The association of increasing tumor vascularity with 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 (4, 13, 14, 24, 25).

In the present study, we investigated angiogenesis in specimens with GS 6 and GS 7, currently the most commonly assigned Gleason scores in prostatic adenocarcinomas (18). Preliminary results showed lower MVD in GS 6 specimens than in GS 7 specimens even

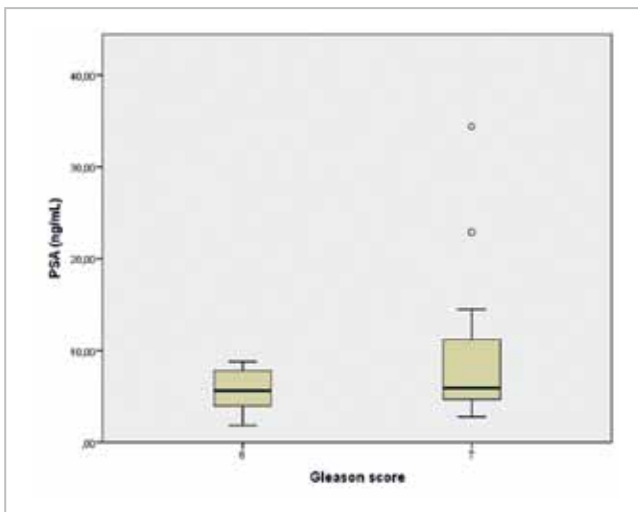


Figure 3. Preoperative PSA level in GS 6 and GS 7 cases (median 5,6 vs. 5,9 ng/mL; $P = 0,9$)

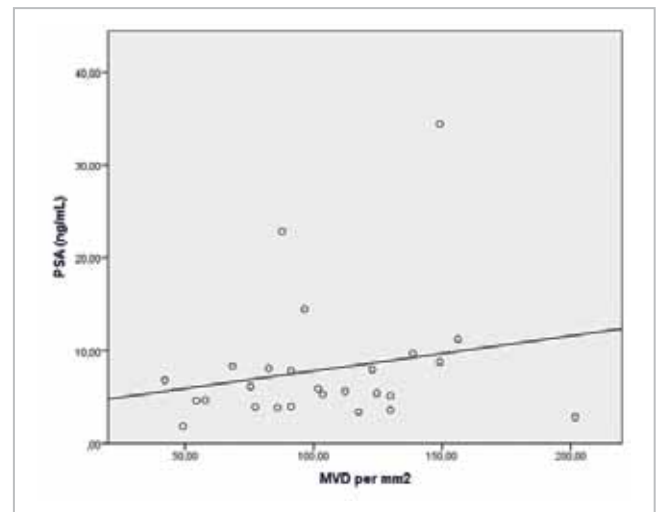


Figure 4. Correlation between PSA level and MVD per mm^2 ($r = 0,20$; $P = 0,30$)

though the difference was not significant. Some authors have shown a correlation between Gleason score and MVD (5–7, 15, 26, 27), whereas other authors did not (28–30). Such discrepancies may be because details of the methodology used to assay MVD, such as the choice of the antibody (e.g., CD31, CD34, von Willebrand factor (vWF), endoglin) have been reported to influence study outcome (31). It was shown that CD31, CD34 and vWF do not stain all microvessels, and particularly not newly formed microvessels (5, 7, 26). Nevertheless several authors used these antibodies (3, 6, 15, 27, 28, 30). 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 for blood vessels in non-neoplastic tissue. Studies also differ with regard to the quantification of angiogenesis. Most authors examined areas of maximal angiogenesis (hotspots) at $\times 200$ magnification (5, 6, 15, 20, 26, 27). Only a few reports determined MVD at $\times 400$ magnification (3, 7, 30). In the present study, we evaluated angiogenesis at $\times 400$ magnification, which allowed more precise quantification of the number of vessels than if we had evaluated MVD at $\times 200$ magnification. Furthermore, in statistical calculations we used two series of data for each specimen: MVD per field and MVD per mm^2 . The difference in MVD between GS 6 and GS 7 specimens in our preliminary report was of borderline statistical significance ($P=0.05$). This was attributable (at least in part) to the small sample size. Therefore, we expect that the final results of our future large-scale study will provide more information about the stage of vascularity in both groups of prostatic adenocarcinoma.

A correlation between MVD and serum levels of PSA was not observed in the present study. This finding is in agreement with those in other reports (26, 27, 29, 30). Conversely, several studies lack information about the association between these two parameters (3, 5, 6, 15, 20, 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, especially in the GS 7 group. PSA is a key variable in prognostic models for clinically localized prostate cancer. It is used to assess pathologic tumor stage and the risk of disease recurrence after local therapy. However, elevation of serum levels of PSA 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 (32). This apparent lack of specificity limits the application of PSA for early detection. However, there is a strong evidence that a cutoff point of 4.0 ng/mL may lead to missing a significant number of cancers (32, 33). Consequently, other PSA-based strategies are being tested for clinical use. These include PSA density (ratio of an individual serum PSA 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 (32). Furthermore, several alternative biomarkers (cell cycle, invasion, cell adhesion, signal transduction, apoptosis, angiogenesis, genetic) have been suggested to supplement (or even replace) PSA to improve strategies for early detection and predict the natural behavior of the tumor (32, 34, 35). Angiogenesis markers indicate that MVD has already been found to be a prognostic factor in several malignancies. However, there remain controversies in prostatic adenocarcinoma due to the small number of such studies (5, 7, 20, 27, 30). Therefore, future studies with large numbers of specimens and a precise methodology of evaluating angiogenesis are needed. If MVD is proved to be an independent prognostic factor in prostatic adenocarcinomas, we believe that it will help to determine which patients may require aggressive adjuvant therapy because of being at high risk for carcinoma recurrence and death.

Presented as a poster at the International Symposium of Clinical and Applied Anatomy, Maribor, Slovenia, July 2011

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