Comparison of microvascular density in cervical carcinoma in relation to predictive pathohistological parameters

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SUMARY

Objective: *The occurrence, development, invasion, and metastasis of tumors are closely linked to angiogenesis, which is reflected by tumor microvessel density. The aim is to analyze microvascular density (MVD) in groups of patients with high grade squamous intraepithelial lesions (H-SIL), and cervical carcinoma, and to compare MVD in relation to the degree of tumor differentiation, size, presence of lymphovascular invasion and lymph node metastases.*

Materials and methods: *The study was retrospective, conducted on histopathological samples of 109 patients who underwent hysterectomy with/without adnexectomy. Patients were divided into two groups depending on the histopathological results: group A - patients with H-SIL, and B with cervical cancer. The control group included surgically treated patients with benign uterine diseases. Based on hematoxylin/eosine staining, representative sample was chosen for immunohistochemistry, and the analysis of CD34 antigen expression and measurement of MVD were done.*

Results: *In order to subdivide groups according to the low (L) and high (H) MVD, in control, group A, and B, with mean MVDs 2.2; 9.85 and 17.19, respectively, a cut-off values were determined. In the control group, LMVD 100% was measured. There were 7 (21.21%) in group A and 29 patients (63.04%) in group B with HMVD. A statistically significant difference was confirmed by comparing HMVD and LMVD in cervical cancer patients with lymph nodes metastasis ($p<0.029$). In the subgroup of patients with other worse pathohistological prognostic factors, a tumor size greater than 2 cm, depth of stromal invasion >10 mm, infiltration of "isthmus" of the uterus, a difference with no statistical significance was confirmed.*

Conclusion: *Invasive cervical cancers are characterised by a significantly higher mean values of MVD compared to H-SIL. Significantly more often, HMVD is associated with the presence of lymph node metastases and histopathological parameters of poor prognosis.*

Keywords: *cervical cancer; microvascular density; angiogenesis; pathohistological parameters*

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INTRODUCTION

Cervical cancer still represents a significant problem, especially in countries without an organized screening program and vaccination, with an estimated 604,000 new cases and 342,000 deaths worldwide in 2020 (1). Tumor angiogenesis is defined as the formation of neovessels from preexisting vascular structures, mainly capillaries and venules, under the influence of a malignant tumor (2,3). Recent studies show the importance of neo-angiogenesis as a key factor that affects the patients' survival and the malignant potential of some gynecological malignant tumors (2). Microvascular density is usually used for the estimation of angiogenesis. In measuring MVD, it is common to label the vessels to be counted. The most frequently used antibodies are those against any of the antigens naturally expressed by endothelial cells such as FVIII, CD31, CD34 and CD105 (4).

It was shown that the status of MVD could be a predicted marker for a worse prognosis in some tumors (4). The high-level of MVD is associated with poor overall survival in cervical cancer patients (3).

MATERIALS AND METHODS

A study was conducted on histopathological (HP) samples of 109 patients who underwent hysterectomy with or without adnexectomy due to a benign disease (myoma), conization due to dysplastic changes or radical hysterectomy due to cervical carcinoma. The data were collected from the Oncology Institute of Vojvodina and divided into two groups:

- Group A (patients who underwent conization after a biopsy confirmed H SIL changes, and the same HP was obtained after conization)
- Group B (patients who underwent a radical hysterectomy after the HP diagnosis of cervical carcinoma)
- Group A obtained 33 patients who had conization due to the H SIL on the cervix. After conization, the material was histopathologically analyzed to determine the grade of dysplasia. The criteria for excluding patients from this group were:
  a. Patients who previously had conization or an ablation of the cervix
  b. Patients with previously diagnosed ginecological malignancy
  c. Patients with L SIL (low-grade squamous intraepithelial lesion) changes

d. Patients with a malignant disease of any other localization

- Group B obtained 46 patients with cervical carcinoma confirmed by a biopsy. After diagnosis and indication, all the patients underwent radical hysterectomy (Piver class III) with bilateral pelvic lymphadenectomy. The materials were histopathologically analyzed with the determination of standard prognostic parameters:
  - Histologic type of tumor
  - Size and depth of stromal invasion
  - Histological grade
  - Presence of lymphovascular invasion
  - Number of extracted lymph nodes
- The Control group obtained 30 patients who had surgery (total hysterectomy) (FIGO stage IB-IIA) due to benign changes of the uterus and/or ovaries. The criteria for excluding the patients from this group were:
  a. Patients who previously had conization or an ablation of the cervix
  b. Patients with a diagnosed precancerous or malignant lesion of the cervix
  c. Patients with a chronic inflammation of the cervix
  d. Patients with a malignant disease of any other localization

  The criteria for excluding the patients from this group were:
  a. Patients with diagnosed cervical carcinoma which was previously treated with chemotherapy or radiation therapy
  b. Patients with previously diagnosed gynecological malignancy
  c. Patients with a malignant disease of any other localization

Immunohistochemistry analyses

Based on H&E (hematoxylin and eosin), stained slydes, representative tissue samples were chosen for immunohistochemistry (IHC). Immunohistochemistry analyses of CD34 antigen expression and measurement of MVD were performed by using the DAKO CD34 M 0823 antibody which marks endothelial cells. In order to obtain precise results, counting of newly formed blood vessels was performed three times, each time without knowing previous count. The number of newly formed blood vessels was determined as the arithmetic mean of previously mentioned counting. Thereafter, we determined the cut-off value based on the obtained arithmetic mean values for MVD control group. A common cut-off value was calculated based on the mean values of Group A and Group B. The obtained results were interpretated as:

- Low MVD: if the arithmetic mean was lower than the cut-off value
- High MVD: if the arithmetic mean was higher than the cut-off value

Statistics

All the statistics were done in Microsoft Excel. The data and results were shown in tables and graphs. Descriptive statistics were used to express the frequence, percent, mean values and standard deviation for statistical data processing. A comparison of the data was performed using the t-test and variance analysis for numericals.

Nonparametric tests, the Pearson chi-square test, the Fischer exact and proportion test were used for attribute features. Statistically significant differences ($p < 0.05$) were marked with an (*), and highly significant differences ($p < 0.01$) with (**).

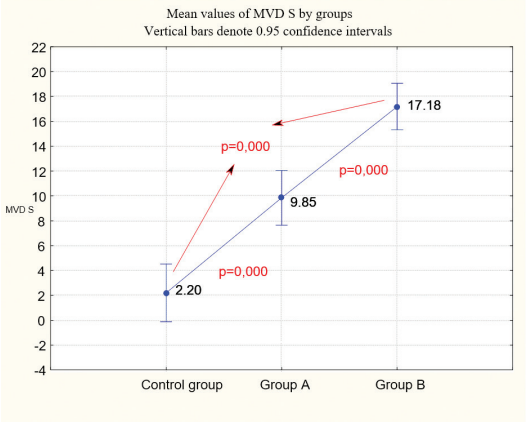
RESULTS

The mean value of the measured microvascular density for the control group was 2.198 ± 1.17, for group A 9.848 ± 1.11 and group B 17.185 ± 0.942. A statistically significant difference was observed by comparing these groups (Table 1; Figure 1; Graph 1).

Table 1. Comparison of mean value of microvascular density between the test groups.

MVD	T	Degree of freedom	Statistical significance
Control group *in relation with* **Group A**	26.63	61	0.0001**
Control group *in relation with* **Group B**	61.64	74	0.0001**
Group A *in relation with* **Group B**	31.64	77	0.0001**

** highly significant statistical difference



Graph 1. Middle values of MVD S by groups.

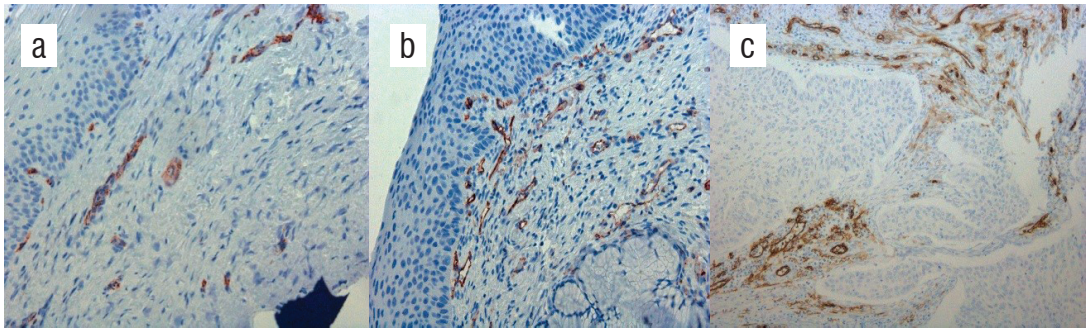
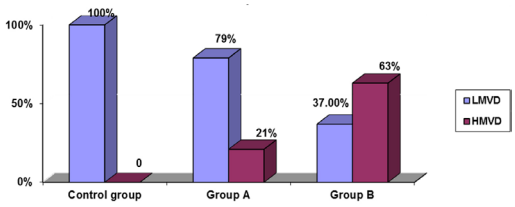


Figure 1. Microvascular density in the control group (a), group A (b) and group B (c). Immunohistochemical identification CD34, LSBA, x200, x100, x100.

After calculating the mean values for the control group (2.2), group A (9.85) and group B (17.19), a cut-off value was determined in order to make a division within the group into low (L) and high (H) microvascular density. The cut-off value for the control group was 2.2, and for the groups A and B was 13.52. In the control group, low microvascular density (100%) was measured in the entire examined material. There were 7 patients (21.21%) with high microvascular density in group A and 29 patients (63.04%) in group B (Graph 2).



Graph 2. The frequency of low and high microvascular density in the test groups.

A statistically significant difference was confirmed by comparing the test groups in relation to the measured microvascular density (Table 2).

Table 2. Comparison of low and high microvascular density between the groups.

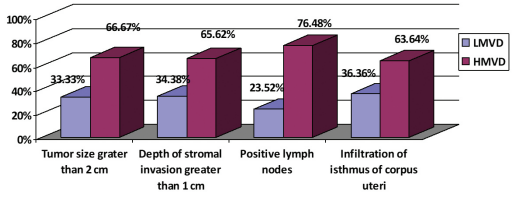
LMVD and HMVD	Pearson Chi-square test	Degree of freedom	Significance p-value
Control group *in relation with* **Group A**	6.484	1	0.01*
Control group *in relation with* **Group B**	28.241	1	0.0001**
Group A *in relation with* **Group B**	13.556	1	0.0002**

*significant statistical difference
**highly significant statistical difference

Considering that very low, insignificant microvascular density with the cut-off value of 2.2 was measured in the control group, the difference between group A and B was further observed.

In the subgroup of 46 patients with worse histopathological prognostic factors, a tumor size was greater than 2 cm (24/46 patients), depth of stromal invasion greater than 10 mm (32/46), positive lymph nodes (17/46), and infiltration of the "isthmus" of the uterus (11/46), with HMVDs 66.67%, 65.62%, 76.48%, 63.64% , and LMVDs 33.33%, 34.38%, 23.52%, 36.36%, respectively, but without statistical significance.

The statistical significance was confirmed in relation to lymphnode positivity ($p<0.029$). Patients with lymphnode metastasis more frequently had HMVD. (Table 3, Graph 3).



Graph 3. Comparison of LMVD and HMVD in the subgroup with poorer pathohistological prognostic factors of the group B.

Table 3. Comparison of LMVD and HMVD with pathohistological parameters in group B.

MVD	LMVD	HMVD	Total (%)	Fisher exact test p-value
	No. (%)	No. (%)		
Tumor size				
Up to 2 cm	9(40.91)	13(59.09)	22(100)	0.4104
Over 2 cm	8(33.33)	16(66.67)	24(100)	
Total	17(36.96)	29(63.04)	46(100)	
Depth of stromal invasion				
Up to 10 mm	6(42.86)	8(57.14)	14(100)	0.4103
Over 10 mm	11(34.38)	21(65.62)	32(100)	
Total	17(36.96)	29(63.04)	46(100)	
Number of lymph nodes				
Up to 10	4(30.77)	9(69.23)	13(100)	0.424
More than 10	13(39.39)	20(60.61)	33(100)	
Total	17(36.96)	29(63.04)	46(100)	
Lymph node metastases				
Positive	4(23.52)	13(76.48)	17(100)	0.029
Negative	13(44.82)	16(55.18)	29(100)	
Total	17(36.96)	29(63.04)	46(100)	
Number of lymph nodes with metastases				
1 positive lymph node	1(25.00)	3(75.00)	4(100)	0.555
2 and more positive lymph nodes	5(38.46)	8(61.54)	13(100)	
Total	6(35.29)	11(63.71)	17(100)	
Degree of histological differentiation				
G1	3(37.50)	5(62.50)	8(100)	p>0.05
G2	10(37.03)	17(62.97)	27(100)	
G3	4(36.36)	7(63.64)	11(100)	
Total	17(36.96)	29(63.04)	46(100)	
Lymphovascular infiltration				
YES	8(44.44)	10(55.56)	18(100)	0.297
NO	9(32.14)	19(67.86)	28(100)	
Total	17(36.96)	29(63.04)	46(100)	
Lymphocytic stromal infiltration				
YES	11(42.30)	15(57.70)	26(100)	0.293
No	6(30.00)	14(70.00)	20(100)	
Total	17(36.96)	29(63.04)	46(100)	
Infiltration of"isthmus"				
YES	4(36.36)	7(63.64)	11(100)	0.627
NO	13(37.14)	22(62.86)	35(100)	
Total	17(36.96)	29(63.04)	46(100)	
Infiltration of parametria				
YES	1(33.33)	2(66.67)	3(100)	0.695
NO	16(37.20)	27(62.80)	43(100)	
Total	17(36.96)	29(63.04)	46(100)	
Infiltration of vaginal "cuff"				
YES	1(25.00)	3(75.00)	4(100)	0.526
NO	16(38.10)	26(61.90)	42(100)	
Total	17(36.96)	29(63.04)	46(100)	

DISCUSSION

Every year cervical cancer is responsible for 342,000 deaths in the world (1). Among them, 87% occur in low-income countries (3). In some studies, a negative correlation between MVD and pelvic lymph node metastases, disease-free survival (DFS) and overall survival (OS) were shown (4,5).

Neoangiogenesis was found to be an important predictive marker for specific biological therapy based on neoangiogenesis inhibitors that inhibit tumor growth and progression (6). Vascular endothelial growth factor (VEGF) has appeared as a major therapeutic target for inhibition (5). The VEGF-targeting monoclonal antibody, bevacizumab, has been approved for the treatment of cervical cancer (6). Neoangiogenesis has an important role for the growth and progression of malignant tumors. Also, angiogenesis is an important mechanism that allows monthly ovulation and the successful realization of pregnancy (5). The method of calculating microvascular density within tumors was developed by Weidner et al. in 1991. The areas with the highest densities of vessels were identified and named hotspots by using light microscopy (7). The value of the MVD in the control group which consisted of patients who had surgery (total hysterectomy) due to benign changes of the uterus and/or ovaries is 2.2. The patients who had conization due to precancerous changes (dysplasia) on the cervix, which belong to group A, showed an MVD mean value of 9.85. Finally, the patients who were diagnosed with cervical carcinoma and belong to group B had an MVD mean value of 17.19. Following that, we determined the cut-off values in order to subdivide groups to LMVD and HMVD. In the control group, all the examined materials showed low microvascular density (100%). There were 7 patients (21.21%) with high microvascular density in group A and 29 patients (63.04%) in group B. Activating a neoangiogenesis in a high grade of dysplasia is an early event that is activated in part to the enhanced expression of the vascular endothelial growth factor by the abnormal epithelium (8). An increase in MVD leads to tumor growth, but it also displays an increased number of tumor cells in vascular space that promotes the hematogenous spread of tumor cells (9).

Not many studies have been done to compare MVD and histopathological parameters such as tumor size, lymph node status, type of tumor, grade, and vascular invasion and they have not shown a significant positive correlation (4,5).

Microvascular density proved to be an important predictor for worse prognosis as the studies presented (10-12).

Cantu De León D et al. compared MVD by the FIGO stadium. The global survival was significantly shorter when MVD was >20, in stages IIA and IIB, but not for IIIB. The authors concluded that MVD plays a role in predicting the recurrence and survival in patients with squamous cell cervical cancer stage II and an age younger than 40 (13). Hu X et al. showed that a high level of MVD was negatively correlated with overall survival (OS) and disease-free survival (DFS) in cervical cancer patients (3). The Wang et al. study assessed the tumor size > 4 cm and advanced FIGO stage as independent unfavorable prognostic indicators of cervical adenocarcinoma cancer specific survival (14). In our study, grade 3 showed the highest percentage of high microvascular density. We can understand similar findings in G2 and G3 when comparing the HMVD based on the number of respondents because in G2 there were 17 patients and in G3 7 patients. Similarly, Aijaz et al. found that MVD distribution was higher in undifferentiated carcinoma compared to differentiated carcinoma and this relationship was found to be statistically significant. The study also confirmed a positive relationship between increased MVD with the chances of metastasis to lymph nodes (15). In our study, the comparison of lymph node metastasis in LMVD and HMVD was positive (LMVD-23.52, HMVD-76.48) and negative (LMVD-44.82, HMVD-55.18) and a statistically significant difference was confirmed. These results can point to MVD as a predicting marker for lymph node metastasis. Deep stromal invasion proved to be another important prognostic factor for cervical cancer. The Sedlis criteria of depth of stromal invasion are expressed as the inner third, middle third, and outer third of cervical wall thickness (16).

In our study, the depth of stromal invasion greater than 10mm comparing LMVD-34.38 and HMVD-65.62 did not show a statistically significant difference. In subgroups of patients with cervical cancer, the worse histopathological prognostic factors showed a higher percentage of HMVD comparing it with LMVD. These results confirmed the importance of neoangiogenesis as a possible predictive factor even though we did not reach the statistical significance that could be a result of our small number of patients in the subgroups and further research is recommended.

CONCLUSION

- Invasive cervical cancers are characterized by a significantly higher mean values of microvascular density compared to H- SIL;
- Significantly more often, HMVD is associated with the presence of lymph node metastases;
- In the group of patients with cervical cancer, it is demonstrated that HMVD is associated with histopathological parameters of poor prognosis;

Conflict of interest

There is no conflict of interest between the author and co-authors.

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