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ORIGINAL ARTICLE

Microtissue density prognostic factor evaluation based on antigens CD34 and CD 105 in ovarian cancer patients

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Abstract

Uncontrollable cell division and disorders of the apoptotic processes constitute the key phenomena in cancer transformation. The theory that the tumour growth above critical density is possible due to creation of the new blood vessels during angiogenesis process was put forward in 1971 by Folkman. The panendotelial antibodies targeted against such markers as CD34 are used most frequently in cancer vessel evaluation. The anti-CD34 reacts with the largest number of endoepithelial cells. The second group constitutes the antibodies that agglomerate with the antigens characteristic for proliferous endoepithelial cells. The most popular marker used for functional endothelial tissues is endoglin called CD105. The subject of this publication is to find the answer to a question whether the practical usage of the CD34 and CD 105 as a prognostic factor in predicting failure of a planned treatment, determining expected remission and the total survival rate is possible. 74 patients with the diagnosed ovarian cancer, treated in the I Clinic of Gynecology Oncology and Gynecology, Medical University in Lublin, between years 1999–2004 were included into the analysis. Representative paraffin blocks with the embedded ovarian cancer fragments were used for immunohistochemical research. Density of the microvessels was being evaluated basing on the expression of the antigen CD34 and CD105. Evaluation of the microvessel density with CD34 and CD105 markers is not useful in forecasting survival rate and disease recurrence in patients with ovary cancer.

Key words

ovarian cancer, CD34, CD105, prognostic factor

INTRODUCTION

Uncontrollable cell division and disorders of the apoptotic processes constitute the key phenomena in cancer transformation. Excessive collections of proliferous cells that create clusters are called primary tumours. The growth of the tumour usually stops when the microtumour is approx. $1mm^3$, i.e. $10^6 – 10^7$ cells. Further growth requires creation of new microvessels supplying such a tubercle [1, 2]. The theory that the tumour growth above critical density is possible due to the creation of the new blood vessels during angiogenesis process was put forward in 1971 by Folkmana et al. [3].

The panendotelial antibodies targeted against such markers as CD34 are used most frequently in cancer vessel evaluation. The anti-CD34 reacts with the largest number of endoepithelial cells. It agglomerates with glicoprotein CD34 responsible for cells adhesion, present on the surfaces of immature hemopoetic and endoepithelial cells [4]. Their main advantage is the possibility of microvessel evaluation on paraffin, as well as frozen scrapes, and identical intenstity of the colour reaction in small and large vessels. These are the properties that allow their usage in regular clinical practice.

The second group constitutes the antibodies that agglomerate with the antigens characteristic for proliferous endoepithelial cells. The most popular marker used for functional endothelial tissues is an endoglin called CD105. THis is homodimeric glicoprotein of the cell membrane, weighing 180kDa [5]. CD105 protein is greatly related to the transforming height factors TGFß1 and TGFß3, which participate in differentiation and proliferation regulation of most types of cells. Combining CD105 with TGF-ß stops protein phosphorylation, whereas the level of its expression modulates the effects of TGF with the antagonistic influence [5].

The CD105 marker has been identified in endothelial tissues of various cancer tumours, while very vague colour reactions with the antibody anti-CD105 have been observed in normal tissue [6]. In solid tumours, the CD105 expression is identified in cancer endothelial tissues localised peripherally and centrolecithally. CD34 has affinity to mature and immature vessels, and CD105 to newly-created vessels [7].

Generally, it is claimed that the histological examination of the microvessels is a standard and relatively effective way of determining vascularisation in cancer tumours.

The objective of the presented study was to find the answer to the question whether the practical usage of the CD34 and CD 105 as a prognostic factor in predicting failure of a planned treatment, determining expected remission and total survival rate, is possible.

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MATERIALS AND METHODS

Clinical characteristics of the examined patients. 74 patients with the diagnosed ovarian cancer, treated in the I Clinic of Gynaecological Oncology and Gynecology at Medical University in Lublin between 1999–2004 were included into the analysis. The average age was 52.6 ± 9.5 (median 51; range 32–77).

In the group, there were 28 women before menopause (37.8%) and 46 after menopause (62.2%). The age of the patients did not influence significantly the histopathological type, or the level of differentiation and clinical severity of ovarian cancer (p>0.05) (Tab. 1).

Table 1. Comparison of age of patients with respect to histopathological diagnosis, the level of differentiation and clinical severity of ovarian cancer.

		Median	25th percentile l	75th percentile	Range	Statistical analysis
Age	Mucous	53.0	48.0	60.0	32.0–69.0	H=1.81 p=0.41
	Endometrial	49.0	47.0	52.0	41.0–70.0	
	Serous	51.5	45.0	59.0	32.0–77.0	
Age	G1	51.0	48.0	55.0	32.0–69.0	H=4.11 p=0.13
	G2	49.0	46.0	54.0	32.0–67.0	
	G3	54.0	47.0	63.5	35.0–77.0	
Age	I	50.0	46.0	55.0	32.0–69.0	H=3.94 p=0.14
	II	54.0	49.0	67.0	44.0–77.0	
	III	53.0	47.0	59.0	35.0–73.0	

In the group of examined patients, serous cancer was the most frequently occuring type of histopathological ovarian malignant neoplasm – 45.9%, and the rarest, endometrial cancer – 23%. Mostly, the low level of histopathological differentiation – G3 (43.2%), and the high level of clinical severity – III (50%) were diagnosed. Radical surgery was performed in 41 patients (55.4%).

Comparison of permanent residence according to histopathological diagnosis is presented in Table 2.

Table 2. Comparison of permanent residence with respect to histopathological diagnosis.

Histopathological Diagnosis		Rural	Urban	Total	Statistical analysis
	Mucous	12 (40%)	11(25%)	23	p=0.54
	Endometrial	8 (26.7%)	9(20.5)	17	
	Serous	10 (33.3%)	24(54.5)	34	
TOTAL		30 (100%)	44(100%)	74	

Comparative analysis based on the Krusal-Wallis test concerning the examined group according to permanent residence did not indicate a significant difference (p=0.54) in the frequency of occurrence of ovarian cancer; however, serous cancer was diagnosed twice as often in the rural patients.

Evaluation of microvessel density, based on antigens CD34 and CD105. expression. Representative paraffin blocks with the embedded ovarian cancer fragments were used for immunohistochemical research. Density of the microvessels was evaluated based on the expression of the antigen CD34 and CD105.

Paraffin scraps weighing 4mm were placed on basic silanised glass. After deparaffinisation and hydratation of the scraps, the antigens mask-off was conducted by heating up scraps in a microwave in citrate bufor of the pH 6.0, 3 × 5 minutes each. The scraps were then incubated in 3% hydrogen dioxide to block the endogenic activity of the peroxidase. During the next stage, the scraps were incubated, respectively, with monoclone mouse antigens aimed against CD34 (DakoCytomation), or CD105 (Novocastra Laboratories) in 1:50 dilution for 30 minutes at room temperature. After finishing each stage, the scraps were rinsed with the TBS solution. Then, a 30-minute incubation was performed with the EnVision™ +/HRP, marked by peroxidase, also at room temperature. Reaction visualisation was obtained by covering the scraps with tetrahydrochloride 3,3'- diaminobenzidine (DAB) at room temperature. During the final stage, the scraps were coloured with Mayer's haematoxiline, dehydrated, enclosed in Canadian balsam, and covered with a microscope slide. For the negative control, mouse control serum was used. The positive control was performed on the scraps coming from a tonsil.

The microvessel density was evaluated in a light microscope, similarly for CD34 and CD105. Firstly, all the preparations were examined at 100× magnification and the 3 fields of the largest microvessel density identified. Then, in these fields, at 400× magnification, the number of microvessels that gave positive immunohistochemical reaction on CD34 and CD105 were examined. The number of microvessels for one high-power field (HPF) indicated the average density of microvessels.

Statistical analysis. Parameter values measured in nominal or ordinal scale were characterised with numerousness and proportion, measured in interval scale using arithmetical average, standard deviation, median, and the range of alternation according to a decomposition form.

To evaluate the existence of differences or correlations between analysed immeasurable parameters, multi-way contingency tables and homogenity or the χ^2 independence tests were used, or in the case of a small number, Yates correction was applied. Due to the skewed layout of measurable parameters evaluated on the basis of the Shapiro-Wilk test, non-parametric tests were used for analyses of the difference existence between examined subgroups. The Mann-Whitney test was used to compare two independent groups while the Kruskala-Wallis test was applied to compare more than two groups, or for multiple comparisons *post-hoc*.

To analyse and compare the survival rate after the surgery, survival analysis was used. The analysis of subgroups according to the examined quality features required tests to compare two or more than two subgroups, i.e. log-rank or F Cox tests. To evaluate the influence of ordinal or constant quantity variables, the Cox proportional hazard model was used.

RESULTS

The average density of microvessels identified with CD34 in the examined group of patients was 48.1/HPF, and the observed values varied within the range of 21.3/HPF – 163.4/HPF. The average value of MVD_{CD105} was 32.6/HPF and the values varied in the range of 10.3/HPF – 100/HPF. Obtained results had rightward skewed layout (Tab. 3).

Table 3. Characteristics of microvessel density, evaluated on the basis of antigens CD34 and CD105 expression.

	Median	25th percentile	75th percentile	Range
MVD_{CD34}	48.1	38.7	76.3	21.3–163.4
MVD_{CD105}	32.6	23.0	100.0	10.3–100.0

The histopathological type of the malignant tumour had a crucial influence on the microvessel density in the case of applying CD34 as well as CD105. The highest average value MVD_{CD34} concerned mucous cancer (MVD_{CD34}=84) and the lowest -endometrial cancer (MVD_{CD34}=40.6). Detailed comparative analysis indicated higher values of MVD_{CD34} in mucous cancer in comparison to endometrial cancer (p=0.0004), as well as serous cancer (p=0.01). In the case of proliferous vessels, the biggest density of microvessels was observed in mucous cancer (MVD_{CD105}=100) and the lowest in serous cancer (MVD_{CD105}=100). The differences between these groups were statistically significant (p=0.002) (Tab. 4).

The level of histopathological differentiation had a crucial impact on the microvessel density in the tumour in the case of marker CD34 as well as CD105. The types of cancer highly differentiated (G1) were characterised by nearly twice as dense microvessels in comparison to poorly differentiated tumours (G3) (81.3 vs. 43.3 for MVD_{CD34}; p=0.03). In the case of proliferous vessels, the differences were much more visible (100 vs.27.6 for MVD_{CD105}; p=0.02) (Tab. 4).

Clinical severity level did not influence significantly the density of microvessels CD34 and CD105 (p>0.05) (Tab. 4).

Evaluation results of MVD_{CD34} and MVD_{CD105} according to histopathological tumour, differentiation and clinical severity level, are presented in Table 4.

Table 4. Characteristics of microvessels density, evaluated on the basis of CD34 and CD105 expression allowing for histopathological diagnosis, differentiation and clinical severity of the ovarian cancer.

		Median	25th percentile	75th percentile	Range	Statistical analysis
CD34	mucous	84.0	48.1	109.3	26.0–163.4	H=15.83 p=0.0004
	endometrial	40.6	33.0	44.3	27.3–98.3	
	serous	48.2	40.6	65.0	21.3–163.2	
CD105	mucous	100.0	31.7	100.0	10.3–100.0	H=11.99 p=0.002
	endometrial	31.0	17.7	34.6	13.7–100.0	
	serous	28.3	20.0	44.0	13.0–100.0	
CD34	G1	81.3	45.3	109.3	21.3–163.4	H=6.99 p=0.03
	G2	48.1	35.0	69.0	27.9–119.7	
	G3	43.3	38.7	58.2	24.3–116.6	
CD105	G1	100.0	27.6	100.0	10.3–100.0	H=8.21 p=0.02
	G2	34.6	30.0	44.0	16.3–100.0	
	G3	27.6	20.0	37.0	13.0–100.0	
CD34	I	56.5	40.6	94.3	21.3–163.4	H=0.91 p=0.63
	II	42.3	27.3	102.0	26.0–162.3	
	III	48.4	41.0	69.7	24.3–109.3	
CD105	I	30.5	24.7	100.0	16.7–100.0	H=0.30 p=0.86
	II	34.3	17.6	100.0	10.3–100.0	
	III	33.3	23.0	100.0	13.0–100.0	

In the comparative analysis of the microvessel density evaluated with markers CD34 and CD105, parameters MVD did not vary among groups according to oncological failure occurrence in a 5-year observation (p>0.05) (Tabs. 5 and 6).

Table 5. Characteristics of microvessel density, evaluated on the basis of CD34 allowing for oncological failures.

MVD_{CD34}		Median	25th percentile	75th percentile	Range	Statistical analysis
Biochemical recurrence	Does not occure	62.5	40.6	92.7	21.3–163.4	Z=1.63 p=0.10
	Occures	45.4	38.7	61.0	26.6–116.6	
Clinical Reccurence	Does not occure	60.7	40.6	93.2	21.3–163.4	Z=1.32 p=0.19
	Occures	47.1	38.7	61.6	26.6–116.6	
5-year survival	Death	48.0	38.7	61.0	26.6–116.6	Z=-0.97 p=0.33
	Survival	56.5	40.6	84.0	21.3–163.4	

Table 6. Characteristics of microvessel density, evaluated on the basis of CD105 allowing for oncological failures.

MVD_{CD105}		Median	25th percentile	75th percentile	Range	Statistical Analysis
Biochemical recurrence	Does not occure	32.8	23.8	100.0	10.3–100.0	Z=0.67 p=0.50
	Occures	32.6	20.7	41.7	13.0–100.0	
Clinical Reccurence	Did not occur	32.8	23.0	100.0	10.3–100.0	Z=0.65 p=0.51
	Occurred	32.6	21.8	40.7	13.0–100.0	
5-year survival	Death	34.3	27.3	44.0	15.0–100.0	Z=0.57 p=0.57
	Survival	31.0	19.0	100.0	10.3–100.0	

On the basis of the Cox model, it can be stated that the quantity parameters of microvessels density MVD_{CD34} and $MVD_{CD105,}$ and the age of the patient, are not significant prognostic factors of a 5-year survival. The obtained results are presented in Table 7.

Table 7. Evaluation of micovessel density impact, based on CD34 and CD105 and age of patients on the total 5-year survival rate.

	Relative risk	P
CD34	0.99	0.16
CD105	1.0	0.78
Age	1.0	0.08

DISCUSSION

Methods which enable determining the level of angiogenesis severity in tumours can constitute an additional prognostic factor in cancer diseases. The number of microvessels evaluated immunohistochemically with the use of a set of endothelial markers can indirectly reflect the intensity of angiogenic processes inside an invasive primary tumour. A relationship between high MVD and survival rate was proved in a set of solid malignant tumours, among others, in endometrial, prostate and cervix cancer [8, 9, 10, 11]. In preclinical analysis, MVD proved to be an effective tool in evaluating the results of experimental anticancer drugs and therapies performed on animal models [12].

The presented study claims that the number of vessels in tumours differs according to histopathological type. The most vascularised tumours were mucous types of cancer. In two papers it was stated that the microvessel density in mucous glandular cancer was the highest among all examined histopathological types [13, 14]. Our own and cited research papers prove the theory that angiogenesis can be induced in various ways, depending on the organ and histopathological type of tumour. Significantly higher values of MVD_{CD34} and MVD_{CD105} were found in G1 level of histopathological differentiation, in comparison to G3. The level of clinical severity did not differentiate considerably CD34 and CD105. It is probable that the angiogenic processes in ovarian cancer are essential in the initial phase of tumour growth, and enable fast enlargement of its volume. These assumptions can be acknowledged by the research conducted by Abulafii et al. [15]. They compared microvessel density in ovarian tumours of women with first grade of disease severity, according to FIGO, with women who had ovarian tumours of borderline malignancy diagnosed after the surgery. The authors stated that the microvessel density in borderline tumours was significantly lower in comparison to other types of malignant ovarian tumours. A number of papers that have been published to date indicate that the prognostic usefulness of microvessel density in maliganat ovarian cancer is controverisal [16, 17, 18]. On the basis of existing data and our own research results, it can be stated that microvessel density indicating an average number of vessels in the microscope field of vision, reflects only the distance between microcapillaries. This distance depends on topical concentration of regulatory angiogenic factors and on their mutual balance [18]. This fact can explain the differences in the observed MVD values in given types of histopathological ovarian cancer. Our own and cited above research results indicate that the level of activity and angigenesis control can differ depending on the histological types of malignant ovarian cancer. In the presented paper, it is claimed that MVD_{CD34} and MVD_{CD105} are not useful for determining the risk of re-occurrence and estimating survival rate in a 5-year observation.

In the presented multifactorial analysis it is stated that MVD_{CD34} and MVD_{CD105} do not constitute essential prognostic factors in patients with ovarian cancer. These results acknowledge the research conducted by Van Diest et al. [19] concerning prognostic value of MVD evaluation in patients with severe ovarian cancer. The authors, using antibody against vWF-VIII factor, stated that microvessel density does not correlate with the severity and cancer differentiation, ploidia of DNA and the proliferation index. Hollingsworth et al. [20], as one of the first researchers, examined the prognostic usefulness of MVD_{CD34} evaluation. They indicated that high microvessel density is connected with shorter remission time and total survival rate.

The team of researchers under the supervision of Abulafii, however, undermined the prognostic usefulness of MVD_{CD34} in patients with primal malignant ovarian tumour [15]. The authors stated that the microvessel density evaluated in peritoneal metastases can be an independent survival prognostic factor. On the other hand, research by Stone et al [21] indicated not only a reverse correlation between microvessel density and total survival rate of patients, but also the relationship of greater vascularisation with a higher level of clinical severity, histopathological differenctiation, and lower chance for optimal cytroreductive surgery [21].

Endoglin (CD105) allows identification of proliferous endotheliocytes and, by the same token, localisation of active neoangiogenesis spots. Kumar et al. [22] compared the prognostic value of CD34 and CD105 markers in a group of 106 patients with breast cancer. The authors stated that the endoglin expression significantly relates to total survival rate and remission time. In the case of CD34, such a relation was not acknowledged [12]. Research by Dales et al. [23] conducted in a group of 900 patients with breast cancer, confirmed the expression of CD105 with the total survival rate and the increase of metastasis occurence risk. In multifactorial analysis, the authors indicated that high endoglin expression constitutes a prognostic factor of survival rate and metastasis occurence risk, independent from other histoprognostic factors, such as size of tumour, differentiation level and its histological type [5].

CONCLUSION

Evaluation of the microvessel density with CD34 and CD105 markers is not useful in forecasting survival rate and disease recurrence in patients with ovary cancer.

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