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Expression of Vascular Endothelial Growth Factors VEGF- C and D, VEGFR-3, and Comparison of Lymphatic Vessels Density Labeled with D2-40 Antibodies as a Prognostic Factors in Vulvar Intraepithelial Neoplasia (VIN) and Invasive Vulvar Cancer

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1. Introduction

Vulvar cancer consists of 2.5-5% of all cancers of the female genital tract. Poland is a country with an average occurrence of this tumor. Most women suffer from this disease between the ages of 60 and 70 years. Recently conducted epidemiological studies indicate that the incidence of intraepithelial neoplasia and vulvar cancer is increasing particularly in women under 50 years of age. The epidemiological model of vulvar cancer in young women involves the role of sexually transmitted infections (sexually transmitted diseases), especially HPV infection with high oncogenic potential, such as HPV 16, 18, 45, 56, 66 and 69; also considered is the importance of habitual smoking in the process of disease genesis.

In older women, vulvar cancer coexists in a high percentage of cases with hyperplasia, lichen sclerosus and squamous cell carcinoma. In 60% of women, vulvar cancer develops in the labia majora, to a lesser percentage of the labia minora, the clitoris and posterior labial commissure. The method of choice in the treatment of vulvar cancer is surgery. Radiation and chemotherapy treatment are usually combined with surgical treatment. Radical excision of the vulva, together with regional lymph nodes, is an operation that involves early and late complications (Judson et al., 2006). Removal of lymph nodes in which metastatic cells are present leads to a reduction in tumor mass, which can have a positive therapeutic effect.

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Cancer metastases present in the lymph nodes removed (lymphadenectomy) is also important for the final classification of the clinical stage of cancer and to decide on how to continue therapy.

Histopathologic assessment of lymph nodes removed in the vulvar cancer operation shows that metastatic cancer cells are found in the I ° stage of cancer in about 16%, grade II clinical stage in about 36%, an average of approximately 28 to 33% of cases. Thus, in approximately 70% of the women operated on with vulvar cancer, whereby there is no evidence of metastasis to the lymph nodes, removal does not improve treatment results (Markowska, 2006). Furthermore, the removal of normal lymph nodes may also have an adverse impact on the local immune status, which is important to the treatment of cancer.

Vulvar intraepithelial neoplasia (VIN) and its classification remains controversial. There are currently three systems of classification VIN:

1. The 3-staged, WHO classification system: VIN1-3
2. The Bethesda system type classification, two-staged, dividing the low-VIN and high degree VIN, and
3. The 2004 established ISSVD classification (International Society for the Study of Vulvovaginal Disease) does not stage VIN. The incidence of VIN has increased in recent decades, while the incidence of invasive vulvar cancer has remained at the same level or even declined in some countries. For example, the U.S. prevalence of VIN3 (vulvar carcinoma in situ) increased by 411% between 1973 and 2000, while the incidence of invasive vulvar cancer increased by only 20% during the same period of time (Judson et al., 2006).

Women with the immunodeficiency virus are approximately four times more vulnerable to HPV infection. However, the incidence of VIN in HIV-positive women ranges from 0.5 to 37% (Kuhn et al., 1999). Thus, a high percentage of HIV infection in women with VIN suggests recommended HIV testing for women with VIN. The lifetime risk of developing invasive cancer in women previously treated for VIN3 is between 2.5-7% (Iversen & Treli, 1998; Jones & Rowan, 1994; Thuis et al., 2000). (Figure 1).

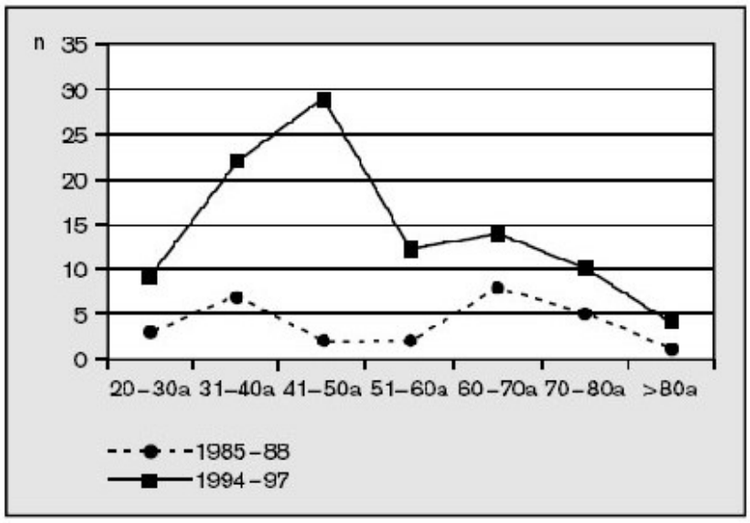

Fig. 1. VIN 2/3 incidence.

VIN is also an independent predictor of relapse (relative risk 3.06) as demonstrated in a study of 101 patients and 33 recurrences Preti (Preti et al., 2000).

In recent years, it has been observed that there is an increased incidence of vulvar cancer in women of younger ages leading to the search for less radical, but also effective surgical methods. These methods have allowed, on one hand, the reduction of injury, reduction of surgery time and reduction in the rate of postoperative complications and importantly, improvement in the quality of life for these women. To achieve this goal, it is equally important to recognize new prognostic factors, among which molecular factors are an attractive model, both in terms of diagnostics and therapeutic potential.

Lymphatic vessels play a key role in the spread of cancer. In recent years, several markers have been identified specific for lymphatic endothelium, which allowed for improved knowledge of the interaction between lymph vessels and lung cancer, but many issues in relation to their prognostic significance remains unclear (Judson et al., 2006; Markowska, 2006).

Proteins belonging to the family of glycoprotein endothelial growth factor (VEGF), referred to as VEGF-C and VEGF-D, are considered the most important regulatory factors in lymphangiogenesis. These factors are potent mitogens to the lymphatic and vascular endothelium. Furthermore, VEGF-C causes an increase in vascular permeability. These regulatory factors are ligands for the receptor VEGFR-3, whose expression is restricted to the endothelium of lymphatic vessels, and in the formation of blood vessels during embryogenesis. Factors VEGF-C and VEGF-D have been identified as stimulators of lymphatic endothelial proliferation, acting through the activation of the receptor 3 VEGF (VEGFR-3), which functions as a specific receptor in mature tissues and shows strong expression within the endothelial cells (Wissmann & Detmar, 2006; Najda & Detmar, 2006). Many clinical studies have shown a positive correlation between expression of VEGF-C and VEGF-D in the primary tumor and lymph node metastases (Donoghue et al., 2007; Nisato et al., 2003).

2. Aim

The aim of the study is to compare the immunohistochemical expression of vascular endothelial growth factors VEGF-C and D, and the expression of VEGFR-3, in VIN and vulvar invasive cancer, and to also compare the density of lymphatic marker D2-40 antibody in both groups, as prognostic factors, and to compare them with other clinicopathologic features.

3. Material and methods

The study was based on tissue material obtained during surgical procedures performed in the Department of Gynecology and Oncology at Jagiellonian University from 2006-2008. This tissue was in the form of cubes stored in paraffin, kept in the archives of the Department of Pathology. The clinical data of patients treated was obtained from the Department of Gynecology and Obstetrics. (Figure 2-6). The material was fixed in formalin on a routine basis. The analysis included 100 cases of vulvar dysplasia (30 - VIN I, 10 - VIN2, 60-VIN3) of which the average age of the patient was 65 years, and 10 cases of vulvar cancer

of which the average age was 71 years. Patients were observed in the Gynecology Oncology Clinic of Jagiellonian University, Krakow from 12 to 48 months. At intervals of 4 - 6 months, patients were evaluated by history and physical examination, undergoing cytologic-colposcopic screening, and assessment of HPV DNA. In case of recurrence, a second operation was performed. The average age of women with vulvar cancer was 71.1 (59-79) years of age and was significantly higher than the average age of women with VIN, which was 56.6 (43-78) years at p = 0.003. (Table 1, Figure 7)

Type	n	Average mean	SD	min	max
VIN3	60	56,0	14,4	43	78
VIN2	10	67,0		67	67
ViN1	30	54,3	3,8	50	57
All groups VIN	100	56,6	11,5	43	78
Ca	100	71,1	7,2	59	79

Table 1. Charakteristics of women studied with VIN and vulvar cancer.

Fig. 2. VIN3 clinical manifestation.

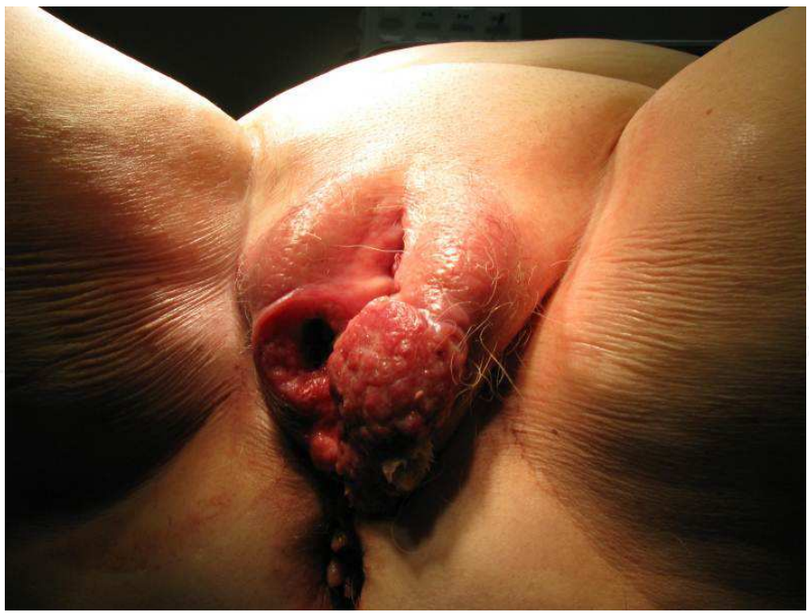

Fig. 3. Vulvar cancer. Clinical presentation.

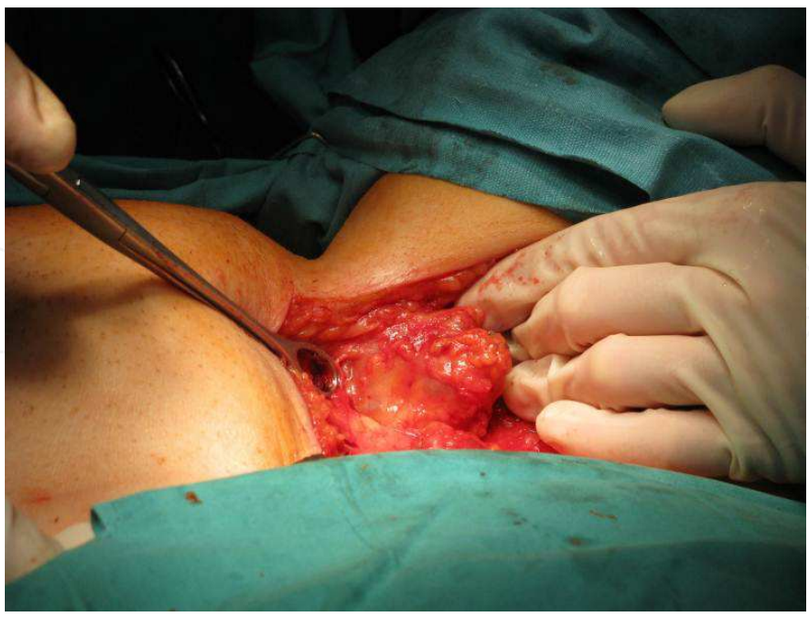

Fig. 4. Metastatic inguinal lymphnodes in vulvar cancer.

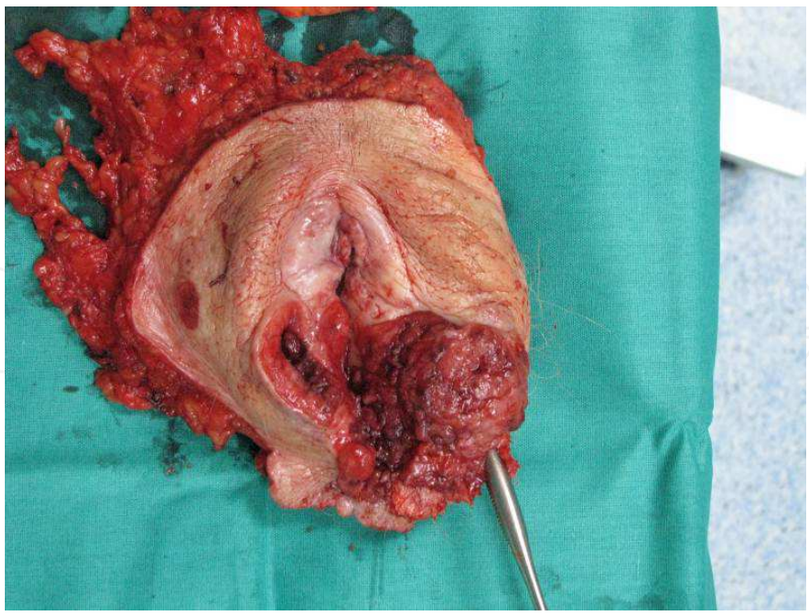

Fig. 5. Postoperative speciemen

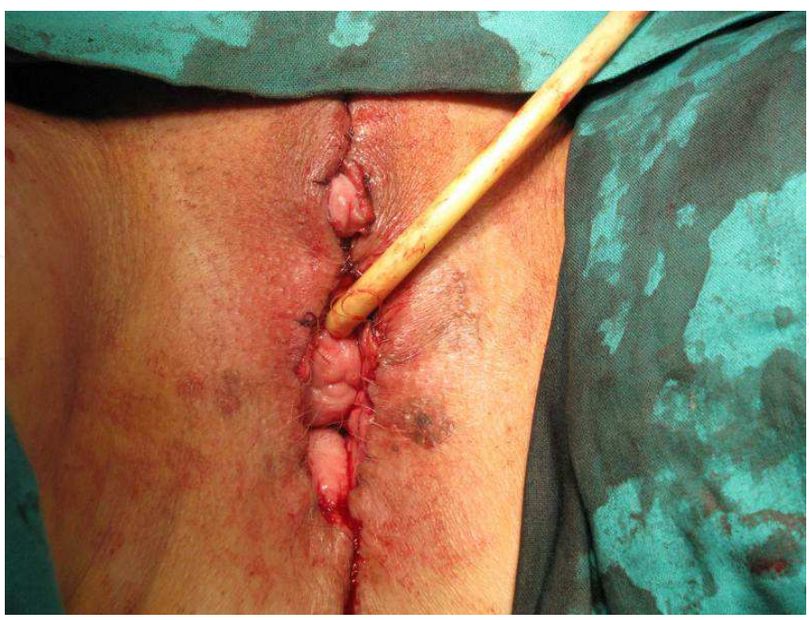

Fig. 6. Clinical presentation after surgery in vulvar cancer.

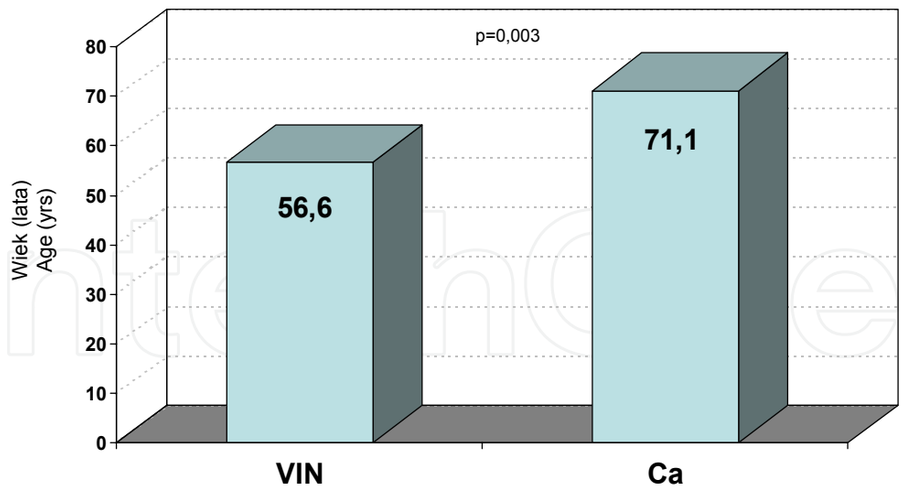

Fig. 7. Age distribution in VIN and vulvar cancer.

The diagnosis of VIN and vulvar cancer was based on histological evaluation of specimens taken from the vulva with guidance of the colposcope. In every case, specimens were also assessed in the presence of the HPV DNA test using Hybrid Capture II (DIGENE Corp.) with the material taken directly from the vulvar brush made of Dacron. The sample was also assessed histologically for metastasis in lymph nodes (superficial inguinal and deep) collected bilaterally in all 100 cases of vulvar cancer and 100 cases of VIN (clinically examined enlarged lymph nodes) during surgical treatment.

For immunohistochemical studies, samples were selected individually and made into paraffin blocks, each representative of a specific case.

From the selected paraffin cubes, 4 microns thick, samples were placed on glass slides coated with silanized basic Super Frost + (SuperFrost Inc.). Deparafinization involved placing the sample for 10 minutes in xylene and then dehydrated lead through three changes of ethyl alcohol of increasing concentration (70%, 86% and 96%), each lasting 5 minutes. In order to inhibit endogenous peroxidase activity, preparations were placed for 10 min in 3% H202 solution. Antigen unmasking was achieved by heating in a microwave oven Whirlpool, 3 times for 5 minutes in a 750W preparation placed in citrate buffer (pH 6.0, 0.01m), or EDTA buffer (pH 8.0, 0.01M).

After incubation, preparations were washed with TBS buffer (50 mM Tris-Hcl, 150 mM NaCl, pH 7.6, DAKO Corporation). To visualize the antigen-antibody complex, we used the En Vision system (DAKO Corporation) and Lab Vision (LabVision) (see the table) with 3-amino-9-ethylcarbazole (AEC) (DAKO Corporation) as a chromogen. Nuclei were contrasted with *Mayer* Hematoxylin for 1 minute and then covered with slide coverslips in glycerol. Basic data on the antibody used in the work is presented in Table 2.

Positive control preparations were: tonsil - for D2-40, placenta- for VEGFR-3 and VEGF-C and VEGF-D, ductal carcinoma of the breast - for VEGF-C and VEGF-D, the small intestine - for D2-40. Negative controls were the same antibody preparations as the original.

Antibody	Type	Clone	Manufacturer	Dilution	Unmasking	Time	Detection system
VEGFR-3	monoclonal	KLT9	Novocastra	1:50	Microwave EDTA, pH=8,0	60 min	Lab Vision
VEGF-C	polyclonal		Santa Cruz	1:100	Microwave EDTA, pH=8,0	12 hrs	En Vision
VEGF-D	monoclonal	78923	R&D systems	1:200	Microwave EDTA, pH=8,0	12 hrs	En Vision
D2-40	monoclonal	D2-40	Covance	Ready-to-use	Microvave citrate buffer ph=6,0	30 min	En Vision

Table 2. Antibodies, their dilution and incubadion time

4. Evaluation of immunohistochemistry

Lymphatic vessel density was evaluated using high power (40x). (Figure 8-10) All D2-40 positive vessels within the area of 0,5mm width beneath the dysplastic epithelium (VIN) or invasive edge of the tumor were counted and the result has been provided. All vessels were counted and the result is given as the number of vessels per 2mm. (Figure 11, 12).

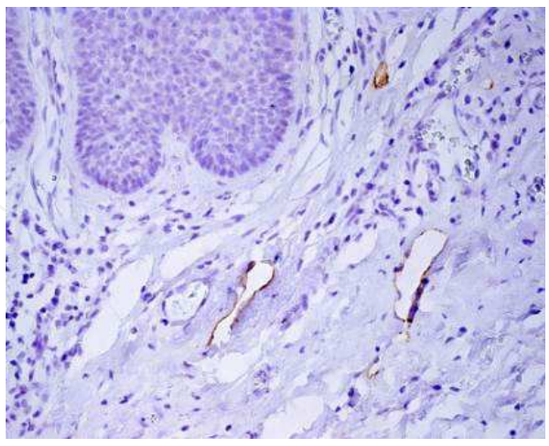

Fig. 8. Histologic picture of VIN I. Immunohistochemical staining with D2-40 antibody. Magnification 40x.

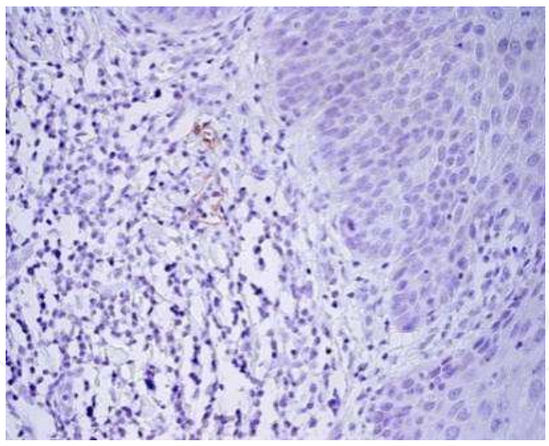

Fig. 9. VIN II . Small lymphatic vessels. Immunohistochemical staining with D2-40 antibody. Magnification 40x.

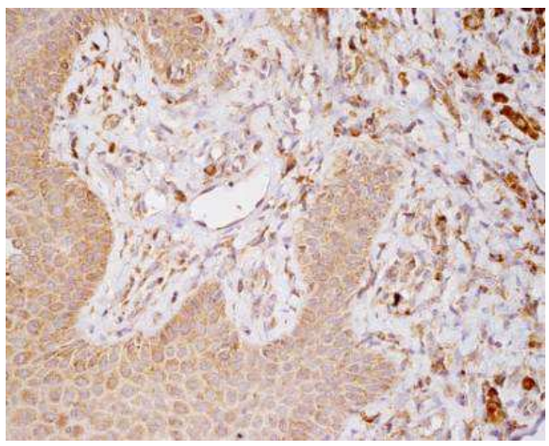

Fig. 10. Histologic picture of VIN. Strong VEGF-D expression in dysplastic epithelium. VEGF-D positive, magnification 40x.

The staining for VEGF-C, VEGF-D, and VEGFR-3 expression was assessed by semiquantitative method, at high (400x) magnification. The severity of expression was evaluated on a scale from 0 to 3; 0-lack of staining, 1-weak, 2-moderate, 3-strong). The number of cells expressing VEGF-C and D and VEGFR-3 were classified to four groups: 0 - no staining or staining in individual cells at the edge of the preparation, 1 - less than 20% positive cells, 2 - 20-50%, 3 - above 50%. Points earned for staining intensity and number of positive cells finally summed 4 groups:

0: 0-1 points
I: 2-3 points
II - 4 points
III - 5-6 points

5. Results

The results are presented in Table 3-10.

DGN	n	VEGF-C	
		0	1
VIN3	60	30	30
		50,00%	50,00%
VIN2	10	10	0
		100,00%	0,00%
VIN1	30	30	0
		100,00%	0,00%
All Grps VIN	100	70	30
		70,00%	30,00%
CA	100	90	10
		90,00%	10,00%

Table 3. Expression of VEGF-C in studied groups of women.

DGN	n	VEGF-D			
		0	1	2	3
VIN3	60	10	20	30	0
		16,67%	33,33%	50,00%	0,00%
VIN2	10	0	10	0	0
		0,00%	100,00%	0,00%	0,00%
VIN1	30	20	1	0	0
		66,67%	33,33%	0,00%	0,00%
All Grps VIN	100	30	40	30	0
		30,00%	40,00%	30,00%	0,00%
CA	100	0	30	60	10
		0,00%	30,00%	60,00%	10,00%

Table 4. Expression of VEGF-D in groups of women studied.

DGN	n	VEGFR-3			
		0	1	2	3
VIN3	60	0	0	60	0
		0,00%	0,00%	100,00%	0,00%
VIN2	10	0	0	0	10
		0,00%	0,00%	0,00%	100,00%
VIN1	30	0	20	10	0
		0,00%	66,67%	33,33%	0,00%
All Grps VIN	100	0	20	70	10
		0,00%	20,00%	70,00%	10,00%
CA	100	10	0	30	60
		10,00%	0,00%	30,00%	60,00%

Table 5. Expression of VEGFR-3 in the studied groups of women.

Identification	n	D2-40			
		Average mean	SD	min	max
VIN3	6	4,88	0,81	4,0	6,0
VIN2	1	3,90		3,9	3,9
VIN1	3	2,00	0,00	2,0	2,0
All Grps VIN	10	3,92	1,49	2,0	6,0
Ca	10	3,80	1,76	1,7	7,0

NS

Table 6. The density of D2-40 vessel in the examined groups of women.

Identification	n	Recurrence		
		NO	YES	
VIN3	6	2	4	
		33,33%	66,67%	
VIN2	1	1	0	
		100,00%	0,00%	
VIN1	3	3	0	
		100,00%	0,00%	
All Grps VIN	10	6	4	
		60,00%	40,00%	
CA	10	7	3	
		70,00%	30,00%	p=0,639

Table 7. Presence of recurrences in examined groups of women.

Indentification	n	Metastasises to lymph nodes		
		NO	YES	
VIN3	6	6	0	
		100,00%	0,00%	
VIN2	1	1	0	
		100,00%	0,00%	
VIN1	3	3	0	
		100,00%	0,00%	
All Grps VIN	10	10	0	
		100,00%	0,00%	
CA	10	8	2	
		80,00%	20,00%	p=0,136

Table 8. Presence of metastasises in examined groups of women.

Identification	n	HPVDNA		
		NO	Yes	
VIN3	6	2	4	
		33,33%	66,67%	
VIN2	1	1	0	
		100,00%	0,00%	
VIN1	3	2	1	
		66,67%	33,33%	
All Grps VIN	10	5	5	
		50,00%	50,00%	
CA	10	6	4	p=0,653

Table 9. Presence of HPV DNA in examined groups of women.

The statistical analysis (statistical package Statistica 8.0, Statsoft. Inc. USA) showed no significant differences in the expression of VEGF-C and D and VEGFR-3 between the VIN group and invasive vulvar cancer group. Weak expression of VEGF-C was found only in two cases of the analyzed series, and in all cases, the expression of VEGF-D and VEGFR-3 was observed. The strongest expression of VEGF-D and VEGFR-3 was observed in the group of invasive cancers. Similarly, the differences in the amount of lymphatic vessels between the group of invasive cancers and VIN group did not reach statistical significance.

The highest density of lymphatic vessels per 2 mm was observed in VIN. In this group, and in most cases, sections of lymphatic vessels were irregular and slightly expanded. In the cancer group, we observed small lymphatic vessels with a narrow, oval lumen. Moreover, in two cases, the presence of lymphovascular space invasion (LVSI) was observed.

The evaluation of recurrence in women treated showed a statistical difference between the group VIN3 and VIN1 at p = 0.058 (ie. 5.8%, with the conclusion that VIN3 and VIN1 differ by the presence of recurrence), which is an interesting trend and needs further investigation in more cases, with specific attention to the literature citing similar occurrence of relapses and VIN1 and VIN2 / 3 (Table 7).

The median survival time without recurrence was the longest in the group of patients with vulvar cancer, in which it was followed by 45.2 months; in women with VIN, it was followed by 37.2 months The shortest survival time without recurrence was among women with VIN3-28.7 months, 95% CI (Table 10). In our opinion, this trend may result from the heterogeneity of women diagnos ed with VIN3, location and multiplicity of the disease.

Group	Median survival time without recurrence	95% CI (95% Confidence Interval)
VIN3	28,7 mos.	14,4 mos. – 43,0 mos.
VIN	37,2 mos.	25,8 mos. – 48,6 mos.
Ca	45,2 mos.	37,6 mos. – 52,8 mos

Table 10. Comparison of average survival time without recurrence in the treated groups of patients.

Disease-free survival curves were compared using log-rank tests. There were no statistically significant differences between survival without recurrence in groups of Ca. and VIN. Two-year survival among patients with VIN was 60%, and for patients with vulvar cancer, 70% (Figure 13). No statistically significant differences in the prevalence of HPV DNA in the test groups and the presence of lymph node metastases in the groin were observed. (Table 8, 9).

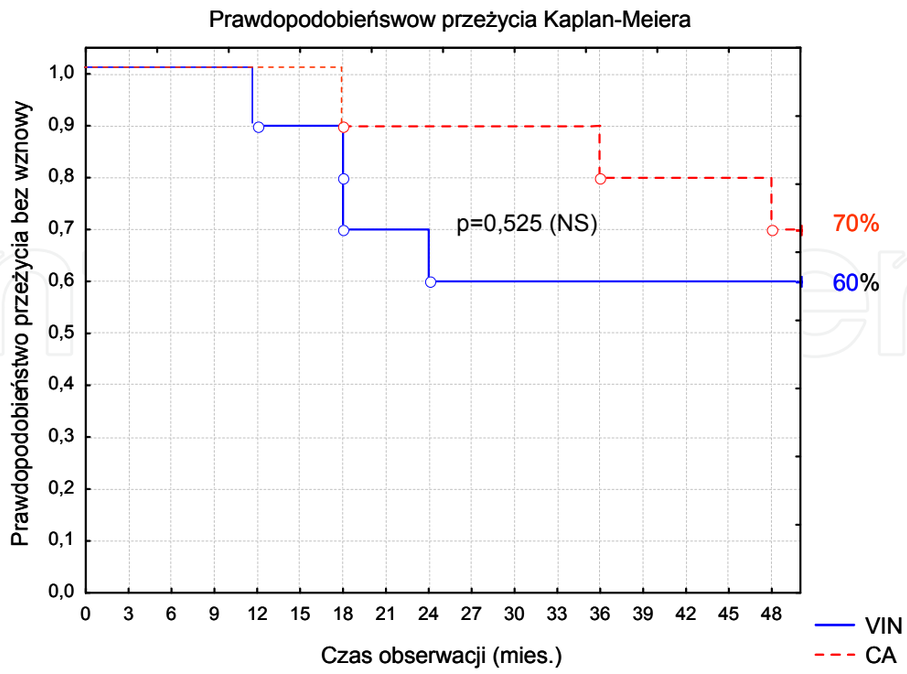

Fig. 13. Recurrence- free survival rate in cancerous and VIN patients.

6. Discussion

Lymphatic vessels play a key role in the spread of cancer. In recent years, several markers specific to lymphatic endothelium have been identified, which allowed for a deeper insight into the relationship between lymph vessels and cancer, although there are still a lot of questions for which there is no clear answer (Hillen & Griffioen, 2007; Sundar & Ganesan, 2007).

The idea that cancer cells spread to already existing lymphatic vessels is still present in the literature, although it seems that there is a belief about the presence of active lymphangiogenesis in tumors (Beasley et al., 2002; Maula et al., 2003; Nathanson, 2003). Currently, though one does not deny the presence of lymphatic vessels in tumors, the problem of the existence of active lymphangiogenesis, and the prognostic value of lymphatic vessel density inside or at the periphery of the tumor has not been completely resolved (Nathanson, 2003; Stacker et al., 2001; Ji, 2006). The functionality of the lymphatic vessels, and their role in metastasis is the subject of discussion (Maula et al., 2003; Ji, 2006; Padera et al., 2002).

In the presence of primary tumors, the extent of lymphangiogenesis can serve as a prognostic indicator of survival. A positive correlation is observed in the density of lymphatic vessels with lymph node metastasis; this has been reported in cases of squamous cell carcinoma of the head and neck (Beasley et al., 2002), gastric carcinomas (Kitadai et al., 2005) and pancreatic tumors (Rubbia-Brandt et al., 2004). In breast cancer, no correlation has been found between the number of lymphatic vessels and lymph node status, to the survival of patients (Bono et al., 2004). The multivariate analysis showed that high-density

peritumoral lymphatic vessels were associated with higher risk of metastasis to lymph nodes in squamous cell carcinomas of the head and neck (Kyzas et al., 2005). In other studies, no correlation was found between the density of lymphatic vessels and lymphatic vessel invasion, lymph node status, and survival of patients as in cases of hepatocellular carcinoma and pancreatic cancer (Mouta Carreira et al., 2001; Sipos et al., 2005). In prostate cancer, or in some case series of breast cancer, there was no opportunity to determine the presence of lymphatic vessels inside the tumor. A lot of work highlights significant changes in the lymph vessels - the proliferation, budding of new blood vessels and expansion in the vicinity of the tumor.

As in physiological conditions, vascular growth factors VEGF-C and-D, activating receptor VEGFR-3, play an essential role in this process. VEGF-C and-D exhibit lymphangiogenic functions through the stimulation of VEGFR-3. They are produced as pre-propeptides that undergo proteolytic processing in the extracellular matrix. Their mature forms exhibit a greater affinity for VEGFR-3, but can also bind VEGFR-2 and induce angiogenesis. Overexpression of VEGF-C and-D in experimental tumor models was accompanied by intensive growth of new lymphatic vessels (Skobe et al. 2001), but in human tumors, these molecules involved in angiogenesis and lymphangiogenesis, are a controversial subject. Some authors state a significant correlation between expression of VEGF-C and-D and lymphangiogenesis and lymph node status, as well as an increase in the density of blood vessels in tumors (Mohammed et al., 2007; Nakamura et al., 2003; Nakamura et al., 2003) although there are also works in which this relationship is not stated (Currie et al., 2004).

In the mouse model of VEGF-C and VEGF-D secreted by tumor cells, there is induced formation of lymphatic vessels in, and around the tumor, which promotes the development of metastasis to regional lymph nodes. These processes have undergone deceleration under the influence of antibodies against VEGFR-3, blocking the activity of the ligands VEGF-D and C (Kitadai et al., 2005). Expression of VEGF-C was observed in many human cancers: breast cancer, cervical and bronchial and prostate and stomach cancers (Roskoski, 2007).

In a study of a small number of patients, including 17 cases of VIN and 26 cases of vulvar cancer, MacLean and colleagues demonstrated the presence of VEGF in 96% of vulvar cancer and only 6% of cases of VIN, not expressing this factor in healthy tissue (MacLean et al., 2000). In experimental models, tumor cells exhibiting overexpression of VEGF-C induced the formation of lymphatic vessels around the tumor (Saharinen et al., 2004; He et al., 2004). An additional issue is the relationship of lymphangiogenesis parameters such as density of lymph vessels, VEGF-C and D, and invasion of lymphatic vessels in tumor progression and prognosis.

In numerous trials, there has been a positive correlation between expression of VEGF-C and the invasion of lymphatic vessels, the presence of lymph node metastases and survival (He et al., 2004). Increased expression of VEGF-D was observed in breast, colorectal, gastric and thyroid multiforme and gliomas, and it has a positive correlation with the presence of lymph node metastases as reported in colorectal cancer, ovarian and bronchus (Roskoski, 2007). In malignant melanoma, VEGF-D likely has an important role both in lymphocytes and angiogenesis (Achen et al., 2001). Similarly, in many tumors, there has been a significant positive correlation between expression of VEGF- C, D in the primary tumor and lymph node status. However, in highly differentiated gastric cancers, this association is not found, and in cases of breast cancer and small cell lung cancer the results were inconclusive.

Increased expression of VEGF-C is a negative prognostic factor in many types of cancer, with the exception of Neuroblastoma, pancreatic cancer and colon cancer. A statistically significant correlation between VEGF-D expression in tumors and shorter overall survival was observed in endometrial cancer, ovarian and pancreatic cancers, in contrast to breast cancer or colorectal cancer (Thiele & Sleeman, 2006). Expression of VEGF-C in tumor cells in many types of cancer generally increases the risk of spread to the lymph nodes, and has some negative effect on survival (Hirakawa et al., 2007).

In gynecological tumors, one also considers the characteristics of lymphangiogenesis. In cervical cancer, a higher density of lymphatic vessels is observed in the periphery of the tumor and in the interior of the tumor in comparison with the normal cervix. The density of blood vessels in the periphery of the tumor correlates positively with higher tumor stage, lymphatic vessel invasion and metastases to lymph nodes, also an independent prognostic factor in multi-and one-dimensional analysis (Gombos et al., 2005). New development of lymphatic vessels has already been concluded in the early stages of carcinogenesis in cervical cancer. Longatto-Filho and colleagues observed that higher density of lymphatic vessels was characterized by changes in invasive cancers (squamous cell carcinoma and adenocarcinoma) compared with changes in preinvasive cancers (CIN I, CIN II, CIN III). However, not found in this study, was a statistically significant correlation between lymphatic vessel density and lymph node status (longatto-Filho et al., 2007). A widely used marker of lymphatic vessels is the D2-40. The expression of this marker was also found in cancer cells and cervical epithelium with features of CIN. Although D2-40 expression in the epithelium did not correlate with clinical features and histological changes, a significant relationship between low expression of D2-40 with invasion of lymphatic vessels and metastases in lymph nodes was observed. This may suggest participation of M2A antigen recognized by D2-40 in the interaction of tumor cells and endothelial cells of lymphatic vessels (Dumoff et al., 2005).

Similarly, expression of vascular growth factors essential for lymphangiogenesis in tumor cells is a phenomenon often described in cases of cervical cancer (Ueda et al., 2001). Increased expression of VEGF-C is attributed to the formation of lymph node metastases (Hashimoto et al., 2001).

A clear and statistically significant difference in the expression of VEGF-C and-D and their receptor VEGFR-3 was observed between (changes in) CIN I and CIN II, CIN III and invasive cancer. A higher degree of dysplasia was associated with increased expression of growth factors and their receptors. This may suggest, in addition to pro-lymphangiogenic activity, autocrine effects of VEGF-C and-D directly on tumor cells via receptor VEGFR-3 (Van Trappen et al., 2003). In cases of vulvar cancer and VIN-type changes described, we see the adverse effect that lymphangiogenic factors have on prognosis and their relationship with the progression of dysplastic lesions (Näyhä & Stenbäck, 2007; Lewy-Trenda et al., 2005). For many years, it was believed that the true VIN3 precursor of invasive vulvar cancer, created a higher risk for women over 40 years of age. Time from VIN3 diagnosis to invasive cancer is estimated to be about 4 years (1.1 to 7.3).

Recently, dominated by many views and defended by clinical data, is the belief that the potential for malignant changes in low grade VIN does not fully reflect the true behavior of

these changes, but rather the survival of patients with this disease. (Rotmensch & Yamada, 2003; Van Seters et al., 2005; Jones et al., 2005). However, there are few reports analyzing the parameters of lymphangiogenesis in these diseases. In the present study, the presence of lymphatic vessels was observed along with changes in the type and degree of dysplasia. It was also found that expression of VEGF-C, VEGF-D and VEGFR-3 in both groups was correlated with the progression of the disease. No statistically significant difference between groups is most likely related to the small size of the analyzed series.

Taking into account the fact that lymph node involvement is an important prognostic factor in vulvar cancer (Raspagliesi et al., 2006), also having been described in other tumor lymphangiogenesis parameters, there is a strong gynecological association with progression and prognosis (Bednarek et al., 2008; Bednarek et al., 2009). One would expect that with vulvar cancer precursors and changes toward progressive disease, it is possible to determine similar relationships. Therefore, also bearing in mind the small number of publications on this matter, it seems that further analysis of lymphangiogenesis in the present group of cases of vulvar cancer is justified and purposeful. Due to the relatively small number of studies that have examined biomarkers (VEGF-C, VEGF-D and VEGFR-3) in carcinogenesis of the vulvar area and the lack of multivariate analysis in these studies, and taking into account the presence of small vulvar cancer and its precursors, one can not establish clear conclusions regarding their prognostic value.

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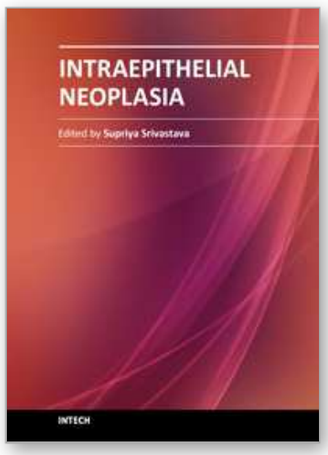

Intraepithelial Neoplasia

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The book "Intraepithelial neoplasia" is till date the most comprehensive book dedicated entirely to preinvasive lesions of the human body. Created and published with an aim of helping clinicians to not only diagnose but also understand the etiopathogenesis of the precursor lesions, the book also attempts to identify its molecular and genetic mechanisms. All of the chapters contain a considerable amount of new information, with an updated bibliographical list as well as the latest WHO classification of intraepithelial lesions that has been included wherever needed. The text has been updated according to the latest technical advances.This book can be described as concise, informative, logical and useful at all levels discussing thoroughly the invaluable role of molecular diagnostics and genetic mechanisms of the intraepithelial lesions. To make the materials easily digestive, the book is illustrated with colorful images.

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