A Comparative Study of Angiogenesis and Lymphangiogenesis as Prognostic Indicators in Epithelial Ovarian Carcinoma

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ABSTRACT

Introduction: Ovarian cancer is the sixth most common cancer and the seventh leading cause of cancer death among women worldwide with 90% of them being of epithelial origin. Its early detection is difficult due to the lack of specificity of clinical symptoms and effective screening procedures, with a 5-year survival rate of ovarian cancer being only 30% approximately. Study aimed to evaluate Angiogenesis and Lymphangiogenesis as Prognostic Indicators in Epithelial Ovarian Carcinoma. IHC evaluation of intra & peritumoral Lymphangiogenesis and Angiogenesis in 25cases of EOC with histological type, grade & stage.

Material and Methods: This was a retrospective study comprising of 25 cases of epithelial ovarian carcinoma diagnosed over a period of 2 years. Quantification of tumor vascularisation by IHC for Lymphangiogenesis was assessed by marker D2-40 and Angiogenesis by CD 34 in 25 patients of Epithelial Ovarian Carcinoma. Morphological aspects such as histological type, grades, stage of cancer as well as vessel distribution that is peritumoral and intra tumoral and vessel density were analyzed.

Result: LVD in peritumoral areas was associated with type, grade & stage of EOC.Cancer induced lymphangiogenesis may be related to microenvironment in tumor infiltrating areas. MVD demonstrated close relation with FIGO stage, grade &type of EOC.

Conclusion: Angiogenesis &lymphangiogenesis may be of significant prognostic value in predicting clinical outcome and future therapeutic measures.

Keywords: Lymphatic Microvessel Density, Angiogenic Microvessel Density, Epithelial Ovarian Carcinoma.

INTRODUCTION

Ovarian cancer is the sixth most common cancer and the seventh leading cause of cancer death among women worldwide with 90% of them being of epithelial origin. Its early detection is difficult due to the lack of specificity of clinical symptoms and effective screening procedures, with a 5-year survival rate of ovarian cancer being only 30% approximately.[1,2]

Epithelial ovarian cancer is one of the most precarious of the various gynecological pathologies.

The best way to tackle the increased mortality rates is by detecting the disease at its earliest clinical stage.[3]

A large number of factors are responsible for increasing the probability of incidence of ovarian tumors. These include genetic and hormonal profiles, ethnic and social factors, fertility, diet, viral infections, increased number of ovulatory cycles, late onset of menopause etc.[4]

Apart from these certain independent factors for instance FIGO stage, residual disease after surgery, histology and lymph node status also play an important role in understanding the natural course process and spread of ovarian cancer.[5]

Ovarian cancer etiology becomes highly multifactoral and thus, like finding for something in a heaped stack, establishing and confirming a single specific biomarker seems to be a very tough occurrence. Keeping these complexities in mind, alternative strategies have to be considered for the purpose of screening and diagnosis.[6-8]

Several panels of biomarkers such as IL13, M-CSF, leptin, prolactin, osteopontin, IGFII, MIF and CA-125 have been used for discrimination of benign and ovarian cancer tissues.

Many biomarkers can be used alone for understanding ovarian cancer mechanisms, these include CA-125, IL13, MIF and M-CSF. Certain biomarkers have also been implicated with the prognosis of ovarian cancer, such as MMP, E-cad and epididymis protein.[9-11]

Various studies also reveal the role of signaling pathways in OVC cell differentiation, cell movement and apoptosis. They are directly associated to OVC tumor suppressor genes and oncogenes.[12]

The ovarian cancer research has mainly put attention on the transformed tumor cell while the role of tumor stroma, especially the fibroblasts which is the main component in stroma has not been studied much. Cancer associated fibroblasts (CAF's) are thought to promote lymphangiogenesis and angiogenesis and in turn contributing to ovarian cancer.[13]

Lymphangiogenesis is the process of lymphatic vessel formation from pre existing post capillary venules. The most important pathological role of lymphangiogenesis is promotion of tumor growth and induction of cancer metastasis.[14]

Physiologically, in mature organisms lymphangiogenesis is activated in very strict conditions e.g tissue repair, inflammation. In pathological cases such as tumor growth (oncogenesis), excessive proliferation and an occurrence of new vessel formation.

Lymphatic spread may be significant in aiding metastasis in ovarian cancer but needs other biological factors also to act in conjunction. Cancer produced lymphangiogenesis is triggered, promoted and executed by many different growth factors such as PROX-1, VEGFR-3, LYVE-1 and Podoplanin(D2-40)[15-17]

D2- 40 or Podoplanin belongs to the family of type-1 transmembrane sialomucin like glycoproteins. It discriminates between lymphatics and blood vessels of ovarian tissue and allows an accurate count that is referred to as lymphatic

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microvessel density.[18]

Angiogenesis is a process of neovascularization. It has an essential role in many physiological processes like organ development, wound healing and tissue regeneration. It also plays an important role in the normal growth and differentiation of cells.[19]

Excessive angiogenesis is associated with pathogenesis, progression and metastasis of malignancies. The presently accepted standard method for quantifying tumor angiogenesis is to assess micro vessel density based on IHC. The most commonly used endothelial marker is CD-34. It is 67kDa transmembrane glycoprotein, located on the surface and directed to the inside part of blood vessel.[20-22]

Many anti-angiogenic agents were developed during last few years therefore it calls for a better understanding of angiogenesis and its role in tumors biology seems to be essential for introduction of new therapeutic strategies.[23]

So the study was done to evaluate Angiogenesis and Lymphangiogenesis as Prognostic Indicators in Epithelial Ovarian Carcinoma. IHC evaluation of intra & peritumoral Lymphangiogenesis and Angiogenesis in 25cases of EOC with histological type, grade & stage.

MATERIAL AND METHODS

This was a retrospective study comprising of 25 cases of epithelial ovarian carcinoma diagnosed over a period of 2 years. Epithelial ovarian carcinoma cases without any treatment till date and not complicated by any other malignancy were included in the study while as cases of primary ovarian cancers other than epithelial carcinoma, metastasis in ovary from any other organ malignancy and any case of AIDS/ TB/ Leprosy were excluded from the study.

Quantification of tumor vascularisation by IHC for Lymphangiogenesis was assessed by marker D2-40 and Angiogenesis by CD 34 in 25 patients of Epithelial Ovarian Carcinoma.

Morphological aspects such as histological type, grades, stage of cancer as well as vessel distribution that is peritumoral and intra tumoral and vessel density were analyzed.

Ovarian tissue obtained was subjected to histopathological processing and paraffin embedding was carried out. The paraffin blocks were subjected to IHC using D2-40 and CD34 markers.

Histopathological evaluation on the basis of type, grade and stage of Epithelial Ovarian Carcinoma was made.

A 4-µm section from one selected paraffin block per subject was stained immunohistochemically with commercially available monoclonal antibodies to D2-40 and CD 34.

Sections were dewaxed briefly in benzene and hydrated in decreasing solutions of alcohols. Antigen retrieval was performed with microwave, using citrate buffer pH6 for 30 minutes. After blocking the endogenous peroxidase, slides were incubated with anti-D2-40 and CD 34 for 30 minutes (ready-to-use, Dako Cytomation, CA, USA). The working system was LSAB-HRP and the final product of reaction was visualized with diaminobenzidine (Dako, Glostrup, Denmark).

Nuclei were stained with Lillie's modified haematoxylin. The entire immunohistochemical procedure was performed with Dako Autostainer Plus (DakoCytomation, Denmark)

Assessment of Vessel density (LVD and MVD): The D2-40 and CD 34 stained section were examined at 100X magnification to delineate "hot spots" i.e. areas of maximum LVD and MVD in intratumoral and peritumoral areas. In three such hot spots in each case all micro vessels (any brown staining endothelial cell clearly separated from adjacent micro vessels, tumor cells or other connective tissue elements was considered to be single countable vessel) were counted at 400X magnification. The average MVD and LVD of three fields in intratumoral and peritumoral area was calculated. Large vessels with thick muscular wall were excluded from the count.

STATISTICAL ANALYSIS

The statistical analysis was performed using the commercially available SPSS software version 17.0. Statistical test was applied to assess the significance of intratumoral and peritumoral difference, expression of D2-40 and CD 34 with type, grade and stage of ovarian carcinoma.

RESULTS

25 cases of malignant epithelial ovarian cancer were reviewed. These cases included 14 cases of serous carcinoma, 7 cases of mucinous and 2 each of endometrioid and clear cell carcinoma. No case of ovarian malignant Brenner was present. 7 cases were of Grade I and II and 11 cases of Grade III. On FIGO staging 5,6,10,4 were of stage I, II, III, IV respectively.

Assessment of d2 – 40 in histological type, grade and stage [table 1,2]

Among the various histological types such as serous, mucinous, endometrioid and clear cell carcinomas, IHC data revealed statistically significant difference in means of intratumoral (p=0.020) & peritumoral (p= 0.045) areas.A significant mean difference was seen in intratumoral area between serous and mucinous carcinoma (p=0.027).A significant mean peritumoral difference was seen between mucinous and clear cell carcinoma (0.049)

Among various grades, significant difference was seen in means of peritumoral (0.002) areas. There was no significant mean difference in intratumoral areas. Mean peritumoral difference was seen between Grade 1 &3 (p=0.002) and 2 &3 (p=0.026).On classifying the cases on the basis of staging into I, II, III and IV stages, significant difference was seen in means of intratumoral (p=0.033) & peritumoral (p= 0.008) areas.Significant mean intratumoral(p=0.033) and peritumoral(p=0.004) difference was seen between stage I & IV.

Assessment of cd34 in histological type, grade and stage [table 3,4]

On the basis of various histological types, i.e. serous, statistically significant difference was seen between the means of intratumoral (p = 0.025) but not in peritumoral among the values. A significant mean difference was seen in intratumoral area between mucinous and clear cell carcinoma. Among the grades, statistically significant difference was seen in means of intratumoral (p = 0.002) and peritumoral (p <0.001). Statistically significant mean intra and peritumoral difference was seen between Grade 1 & 3 and Grade 2 &3. Between various stages, statistically significant difference was seen in means of intratumoral between Stage I & IV

S No	Histological type	No. of cases(N)	Intramural	Pertumoral
1.	Serous Carcinoma	N	14	14
2.	Mucinous Carcinoma	N	7	7
3.	Endometrioid Carcinoma	N	2	2
4.	Clear cell Carcinoma	N	2	2
	Total	N	25	25
		Mean	9.32	7.96
		SD	2.78	2.73
		p- value	.020	.045
	Between Groups(ANOVA)		Intratumoral	Peritumoral
		p- value	.020	.045
	Serous vs Mucinous	Mean Difference	3.357	1.790
		p- value	.027	.403
	Serous vs Endometrioid	Mean Difference	1.210	2.357
	Serous vs Clear cell	p-value	.904	.580
	Mucinous vs Endometrioid	Mean Difference	1.786	3.643
	Mucinous vs Clear cell	p-value	.751	.223
	Endometrioid vs Clear cell	Mean Difference	2.143	.571
		p-value	.675	.991
		Mean Difference	5.143	5.429
		p-value	.058	.049
		Mean Difference	3.000	6.000
		p-value	.591	.093

Table-1: D2 40 Assessment on the basis of histological types

Grades	No. of cases	Intratumoral	Peritumoral
1	N	7	7
	Mean	7.71	5.86
	SD	2.690	1.676
2	N	7	7
	Mean	9.14	7.00
	SD	3.436	2.309
3	N	11	11
	Mean	10.45	9.91
	SD	1.968	2.256
Total	N	25	25
	Mean	9.32	7.96
	SD	2.780	2.731
Between Groups (ANOVA)	p- value	.121	.002
1vs 2	Mean difference		1.143
	p- value		.582
1 vs 3	Mean difference	NA	4.052
	p- value		.002
2vs 3	Mean difference		2.909
	p-value		.026

Table-2: D2 40 Assessment among grades and stages

DISCUSSION

Concerning the complexity of mechanisms responsible for carcinogenesis, there are still no routine methods of early detection of ovarian cancer, and its biological behavior.

Clinical stage, histological grade and the type of neoplasm are the most significant prognostic factors of ovarian cancer. It is essential to investigate new biomarkers which allow for a better prognosis of patients with ovarian neoplasms.[24,25]

As tumor mushrooming is severely limited by nutrient supply to the proliferating tumor cells, angiogenesis plays a crucial role in tumor growth and metastasis. Tumor genesis of malignant neoplasm is associated with extensive neovascularization for tumor growth progression.[26]

Cancers after the so called angiogenic switch acquire the ability to induce new vessel formation. Neovascularization depends on the ability to produce specific factors stimulating and inhibiting new blood vessel formation. These factors can be released by neoplastic cells, stromal components and immune cells like macrophages. Angiogenesis is assessed by micro vessel density which can be evaluated after immunostaining endothelial cells with antibodies against CD 31, CD 105 and CD 34 for blood vessels.[27,28]

CD34 is a highly glycosylated transmembrane protein which is expressed on immature hematopoietic cells as well as on luminal endothelial cells. Tanigawa et al;1996 reported that CD34 displayed a better sensitivity and specificity than FVIII for endothelial cells induced by tumor angiogenesis.[29]

In an embryo, vessels are derived form in situ differentiation of undifferentiated precursor cells to vascular endothelial cells (Risau, 1997). Subsequently, this primeval structure expands by sprouting of capillaries from pre-existing vessels or intussusception, in which interstitial tissue such as tumor cells are integrated into the lumen of pre-existing vessels (Carmeliet, 2000). In addition, tumor cells next to existing vessels have an ability to form a perivascular cuff (Yancopoulos et al., 2000). It is still a controversial question whether these vessels result from tumor cells invading lumen, from 'vasculogenic mimicry' of tumor cells, or from exposing underlying tumor cells due to apoptosis of endothelial cells.[30-32]

Among the angiogenesis markers most commonly examined is the microvessel's density (MVD). During our study we assessed MVD using IHC marker CD34. A study carried out by Radoslaw B et al in 2011; revealed that the mean value of MVD was significantly higher in serous ovarian carcinoma than benign

Sr. No.	Histological type	No. of	Intratumoral	Peritumoral
1.	Serous Carcinoma	N Mean (MVD) SD	14 8.29 2.37	14 8.29 3.15
2.	Mucinous Carcinoma	N Mean SD	7 6.43 3.64	7 8.14 2.73
3.	Endometrioid Carcinoma	N Mean SD	2 7.50 3.54	2 7.00 1.41
4.	Clear cell Carcinoma	N Mean SD	2 14.00 .00	2 13.50 .71
	Total	N Mean SD	25 8.16 3.26	25 8.56 3.11
	Between Groups (ANOVA)		Intratumoral	Peritumoral
		p- value	.025	.115
	Serous vs Mucinous	Mean Difference	1.857	NA
		p- value	.495	
	Serous vs Endometrioid Serous vs Clear cell Mucinous vs Endometrioid Mucinous vs Clear cell Endometrioid vs Clear cell	Mean Difference p-value Mean Difference p-value Mean Difference p-value Mean Difference p-value	.786 .982 5.714 .060 1.071 .963 7.571 .014 6.500 .126	NA

Table-3: CD 34 Assessment on histological types of epithelial ovarian tumors

serous adenomas. Also, no correlation was observed between MVD and FIGO staging, but differences between MVD and tumor grade were on a statistical borderline (p=0.07).[33]

In our study we observed a statistical difference in intratumoral(p=0.002) and peritumoral (p<0.001) areas of ovarian cancer among grades 1 & 3 and 2 & 3. In a study done by Lei He et al; 2015, it was seen that both overall survival and progression free survival were significantly poorer with high MVD than with low MVD in ovarian cancer patients. In our study we found a significant mean difference in intratumoral areas in epithelial ovarian carcinoma(p= 0.025)[34]

Shelly Sehgal et al; 2013 in a study consisting of 42 cases of ovarian surface epithelial tumors showed that MVD was much higher in malignant ovarian tumors along with intense CD34 expression. In our study we found that the expression of CD34 on endothelium cells correlated with histological examination is an indicator for clinical prognosis and survival rate.[35]

In postnatal life under normal conditions, lymphangiogenesis is a quiescent process, but an active formation of new lymphatics was reported in various neoplastic and non-neoplastic human diseases. Although the lymphatic vessels invasion is unanimously accepted as a poor prognostic factor, the early steps of invasion by tumor cells and their clinical significance are still a matter of debate.

Lymphangiogenesis is a complex multistep process, brought about mainly by vascular endothelial growth factor-C and its cognate receptor. The binding of the ligand to the receptor stimulates endothelial cells of the postcapillary venules to acquire lymphatic phenotype and, finally, they lose the connection with blood vessels.[36,37] The study of lymphatic vessels had been hampered with difficulty due to the overlapping morphological features between blood and lymphatic endothelial cells.[38] By immunochemistry, lymphangiogenesis had been reported in many solid tumors, not only in peritumoral but also intratumoral.[39]

A certain specific antibodies were introduced, that can discriminate between blood vessels and lymphatic vessel endothelium such as LYVE-1, podoplanin (D2 40), Prox-1, desmoplakin and VEGFR (Witte MH et al. 2006) Due to these antibodies, it was possible to count LVs in the peritumoral and tumoral area, procedure known as lymphatic microvessel density (LMVD). LMVD was calculated in almost all human tumors and specially in carcinomas, and in most of the cases, a positive correlation between LMVD, lymph node metastases, and prognosis was found.[40,41]

Podoplanin/D2-40 belongs to the family of type-1 transmembrane sialomucin-like glycoproteins. It can be used as a marker of lymphatic endothelial cells. In the normal ovary, primary and secondary ovarian follicles show strong podoplanin expression and the reaction becomes negative in the luteal body and albicans body. A role for podoplanin was suggested in the early differentiation of the granulosa cell layer of the ovarian follicle.[42]

In our study we assessed LVD in epithelial ovarian cancers by D2-40 for lymphatic vessels. In a study done by Schacht V et al. 2005; podoplanin expression was found in 4 of 4 cases of

Grades		Intratumoral	Peritumoral
	N	7	7
	Mean	6.57	6.29
	SD	2.23	1.70
2	N	7	7
	Mean	6.00	6.86
	SD	2.24	1.95
3	N	11	11
	Mean	10.55	11.09
	SD	2.88	2.55
Total	N	25	25
	Mean	8.16	8.56
	SD	3.26	3.11
Between Groups (ANOVA)	p- value	.002	<0.001
1vs 2	Mean difference	.571	.571
	p- value	.908	.877
1 vs 3	Mean difference	3.974	4.805
	p- value	.010	<0.001
2 vs 3	Mean difference	4.545	4.234
	p-value	.003	.002
Stages			
Stage		**Intratumoral**	**Peritumoral**
I	N Mean SD	5 6.20 2.28	5 6.00 1.58
II	N Mean SD	6 6.33 2.66	6 8.67 2.66
III	N Mean SD	10 8.90 2.73	10 9.00 2.91
IV	N Mean SD	4 11.50 3.79	4 10.50 4.51
Total	N Mean SD	25 8.16 3.26	25 8.56 3.11
Between Groups (ANOVA)	p-value	.026	.158
TI vs II	Mean Difference p-value	.133 1.000	NA
I vs III	Mean Difference p-value	2.700 .323	
I vs IV	Mean Difference P-value	5.300 .048	
II vs III	Mean Difference P-value	2.567 .316	
II vs IV	Mean Difference P-value	5.167 .044	
TIII vs IV	Mean Difference P-value	2.600 .420	

Table-4: CD 34 assessment on the basis of grades & stages

dysgerminoma and in one of 3 cases of granulosa cell tumor.[43] Mishima K et al. 2006; suggested a role of podoplanin in invasion and metastasis. This was based on the observation that an increased expression of podoplanin was consistently correlated with the presence of metastasis. It was reported in 2006 by Wicki A et al. that podoplanin expressing cells were present in more than 80% of human squamous cell carcinomas at the invasion front.[44]

In a study carried out by Laurentiu P et al. 2011; lymphatic vessels were found not only in stroma but also in tumor area. Also a strong correlation was found between the intratumoral lymphatic microvessel density and the stage of ovarian cancer.[45] In our study we found that D2-40 expression on the basis of stage was found in peritumoral area between stage 1 and 2 of ovarian cancer. In a study conducted by Shouhua Yang et al, 2016 it was found that lymphatic vessels density in epithelial ovarian cancer was not related to clinical stage, pathological types, cell differentiation of tumor cell while as LVD was positively related to LVI, LVP and the volume of ascites. These results indicated that the proliferation of lymphatic vessels in EOC induced the progression to advanced epithelial ovarian cancer via metastasis.[46]

Lichun Li et al; 2009 in their study showed intratumoral lymphatics as independent prognostic indicators. Intratumoral lymphatics are associated with lymphatic invasion and thus promote malignant progression in ovarian carcinomas.[47]

In our study, the D2-40-stained endothelial cells with a lumen were defined as individual lymphatic vessels. We observed a significant correlation between the lymphatic density and in means of intratumoral and peritumoral among the various histological types. Incase, of assessment of D2-40 in grades, a significant difference is seen in means of peritumoral areas of epithelial ovarian carcinoma.

Lymphatic vessels spread may be significant in aiding metastases in ovarian cancer but requires other biological factors to act in conjunction, as it does not have clear-cut prognostic significance.

CONCLUSION

LVD in peritumoral areas was seen to be significantly associated with type, grade and stage of malignant epithelial ovarian lesions (EOC). This indicates a proliferation of lymphatic vessels in EOC in peritumoral areas possibly induces(shows) a progress to advanced epithelial ovarian cancers. Thus, a cancer induced lymphangiogenesis may be related to the microenvironment in tumor infiltrating areas(peritumoral areas) and hence, result in induction of cancer metastasis.

Markers of lymphangiogenesis might be useful in both prognosis as well as for targeted treatment on neoplasm's. Lymph vessels are an important component of tumoral stroma and are also responsible for creating conduits for tumor metastasis.

MVD demonstrated a close relation with FIGO stage, grade & type of EOC. MVD in our study can be correlated significantly with type, grade and stage in case of intratumoral areas. High level of MVD might be an independent prognostic factor. This suggests that vascularisation is important for tumor growth.

A close relation between tumor vascularisation and its growth dynamics suggests that angiogenesis markers may be of significant prognostic value in predicting the clinical outcome and development of gynaecological malignancies and can be useful in predicting the result of planned treatment and as targets for future therapeutic measures.

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REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136: E359-86.
2. Lengyel E. Ovarian cancer development and metastasis. Am J Pathol. 2010;177:1053-1064.
3. Timmerman D, Testa AC, Bourne T. International Ovarian Tumor Analysis Group. Logistic regression model to distinguish between the benign and malignant adnexal mass before surgery: a multicenter study by the International Ovarian Tumor Analysis Group. J Clin Oncol. 2005;23:8794-801.
4. Jelovac, D., Armstrong, D.K. Recent progress in the diagnosis and treatment of ovarian cancer. A Cancer Journal for Clinicians. 2011;61:183–203.
5. Lei He. Microvessel density as a prognostic factor in Ovarian Cancer: a systematic review and meta analysis. Asian Pac J Cancer Prev. 2015;3:869-874.
6. Jazaeri A. Molecular profiles of hereditary epithelial ovarian cancers and their implications for the biology of this disease. Molecular Oncology. 2009;3:151–156.
7. Bast, R. C., Jr., Hennessy, B., & Mills, G. B. The biology of ovarian cancer: new opportunities for translation. Nature Reviews Cancer. 2009;9:415–428.
8. Moore, L.E., Pfeiffer, R.M., Zhang, Z., Lu, K.H., Fung, E.T., Bast, R.C., Jr. Proteomic biomarkers in combination with CA 125 for detection of epithelial ovarian cancer using prediagnostic serum samples from the prostate, lung, colorectal, and ovarian (PLCO) cancer screening trial. Cancer. 2012;1:91–100.
9. Ripley, D., Shoup, B., Majewski, A., & Chegini, N. Differential expression of interleukins IL-13 and IL-15 in normal ovarian tissue and ovarian carcinomas. Gynecologic Oncology. 2004;92:761–768.
10. Suzuki, M., Ohwada, M., Aida, I., Tamada, T., Hanamura, T., & Nagatomo, M. Macrophage colony-stimulating factor as a tumor marker for epithelial ovarian cancer. Obstetrics and Gynecology. 1993;82:946–950.
11. Visintin, I., Feng, Z., Longton, G., et al. Diagnostic markers for early detection of ovarian cancer. Clinical Cancer Research. 2005;14:1065–1072.
12. Li, A. J., Baldwin, R. L., & Karlan, B. Y. Estrogen and progesterone receptor subtype expression in normal and malignant ovarian epithelial cell cultures. American Journal of Obstetrics and Gynecology. 2003;189:22–27.
13. Zhang Yuan. Cancer Letters. 2011;303:47–55.
14. Iwona W. Zdr Publ. 2015;1:24-28.
15. Li S. Cancer stem cells, lymphangiogenesis, and lymphatic metastasis. Cancer Letters. 2015;357:438-47.
16. Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. Cell. 2011;146:873-87.
17. Omachi T, Kawai Y, Mizuno R. Immunohistochemical demonstration of proliferating lymphatic vessels in colorectal carcinoma and its clinicopathological significance. Cancer Lett. 2007;1-2:167-72.
18. Suzuki-Inoue K, Kato Y, Inoue O. Involvement of the snake toxin receptor CLEC-2 in podoplanin-mediated platelet activation by cancer cells. J Biol Chem. 2007; 282:25993-6001.
19. V. Kumar, A. K. Abbas, N. Fausto. Robbins and Cotran Pathologic Basis of Disease. 2010.
20. Raica M, Cimpean AM, Ribatti D. Angiogenesis in pre-malignant conditions. Eur J Cancer. 2009;45:1921-34.
21. Nagy VM, Buiga R, Brie I, Todor N, Tudoran O, Ordeanu C et al. Expression of VEGF, VEGFR, EGFR, COX-2 and MVD in cervical carcinoma, in relation with the response to radiochemotherapy.Rom J Morphol Embryol. 2011; 52:53–59.
22. Czekierdowski A, Czekierdowska S, Czuba B, Cnota W, Sodowski K, Kotarski J et al. Microvessel density assessment in benign and malignant endometrial changes. J Physiol Pharmacol. 2008;4:45–51.
23. Dharmalingam P, Roopesh Kumar VR, Verma SK. Vascular endothelial growth factor expression and angiogenesis in various grades and subtypes of meningioma. Indian J Pathol Microbiol. 2013;56:349-54.
24. Raica M, Cimpean AM, Ribatti D. Angiogenesis in pre-malignant conditions. Eur J Cancer. 2009;45:1924–1934.
25. Rudlowski C, Pickart AK, Fuhljahn C, Friepoertner T, Schlehe B, Biesterfeld S et al. Prognostic significance of vascular endothelial growth factor expression in ovarian cancer patients: a long-term follow-up. Int J Gynecol Cancer. 2006;16:183–189.
26. Bamberger ES, Perrett CW. Angiogenesis in epithelial ovarian cancer. Mod Pathol. 2002;55:348-359.
27. S. Sharma, M. C. Sharma, and C. Sarkar. "Morphology of angiogenesis in human cancer: a conceptual overview, histoprognostic perspective and significance of neoangiogenesis. Histopathology. 2005;46:481–489.
28. P. B. Vermeulen, G. Gasparini, S. B. Fox. Second international consensus on the methodology and criteria of evaluation of angiogenesis quantification in solid human tumours. European Journal of Cancer. 2002;38:1564–1579.
29. Tanigawa N. Cancer Lett. 1996;2:281-90
30. Risau W. Mechanisms of angiogenesis. Nature. 1997;6626:671-4.
31. Carmeliet P. Curr Atheroscler Rep. 2000;5:407-16.
32. Yancopoulou GD. Pediatr NeuroSurg. 2000;1:49-55.
33. Radoslaw B. Advances in Clinical and Experimental Medicine. 2011;20:737–743.
34. Lei He. Microvessel density as a prognostic factor in Ovarian Cancer: a systematic review and meta analysis. Asian Pac J Cancer Prev. 2015;3:869-874.
35. Shelly Sehgal. J Interdiscipl Histopathol. 2013;3:145-152.
36. Achen MG, Jeltsch M, Kukk E. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (FLT4). Proc Natl Acad Sci USA. 1998;95:548-53.
37. Witte MH, Jones K, Wilting J. Structure function relationships in the lymphatic system and implications for cancer biology. Cancer Metastasis Rev. 2006;25:159-84.
38. Yang S, Cheng H, Cai J, Cai L, Zhang J, Wang Z. PlGF expression in pre-invasive and invasive lesions of uterine cervix is associated with angiogenesis and lymphangiogenesis. APMIS. 2009;117:831-838.
39. Cheng D, Liang B, Li Y. Serum vascular endothelial growth factor (VEGF-C) as a diagnostic and prognostic marker in patients with ovarian cancer. PLoS One. 2013;8:55309.
40. Witte MH, Jones K, Wilting J. Structure function relationships in the lymphatic system and implications for cancer biology. Cancer Metastasis Rev. 2006;25:159-84.
41. Shields JD, Borsetti M, Rigby H. Lymphatic density and

metastatic spread in human malignant melanoma. Br J Cancer. 2004;90:693-700.
42. Suzuki-Inoue K, Kato Y, Inoue O. Involvement of the snake toxin receptor CLEC-2 in podoplanin-mediated platelet activation by cancer cells. J Biol Chem. 2007; 282:25993-6001.
43. Schacht V, Dadras SS, Johnson LA. Up-regulation of the lymphatic marker podoplanin, a mucin-type transmembrane glycoprotein, in human squamous cell carcinomas and germ cell tumors. Am J Pathol. 2005;166:913-21.
44. Mishima K, Kato Y, Kaneko MK. Podoplanin expression in primary central nervous system germ cell tumors: a useful histological marker for the diagnosis of germinoma. Acta Neuropathol. 2006;111:563-8.
45. Laurentiu P. Clinical Significance of Lymphatic Microvessel Density and D2-40 Immunohistochemical eExpression in Ovarian Cancer. TMJ. 2011;61:1-2.
46. Shouhua Yang. Lymphangigenesis in human epithelial ovarian cancer is related to the formation of ascites. Int J Clin Exp Pathol. 2016;2:1660-1667.
47. Li, L., Liu, B., Li, X., Yang, S., Xiao, J., Chen, M et al. Vascular Endothelial Growth Factor D and Intratumoral Lymphatics as Independent Prognostic Factors in Epithelial Ovarian Carcinoma. Anat Rec. 2009;292:562–569.

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