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MICROVESSEL DENSITY ASSESSMENT IN BENIGN AND MALIGNANT ENDOMETRIAL CHANGES

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Tumor angiogenesis is believed to be a prognostic indicator associated with tumor growth and metastasis. Microvessel density (MVD) assessment with common endothelial markers such as CD34 has been found to influence prognosis among endometrial carcinoma patients. The CD105/endoglin antibody has been reported to preferentially bind to proliferated endothelial cells in tissues participating in angiogenesis. The aim of this study was to evaluate the quantification of angiogenesis by assessing MVD in endometrial lesions when comparing the performance of anti-CD34 and anti-CD105 in women with benign and malignant endometrial changes. The study included 58 women (37 postmenopausal) with normal, hyperplastic and malignant endometrium in which preoperative transvaginal sonography was performed. Histological results of the removed endometrium were correlated with MVD assessed in “hot areas” where high densities of microvessels were detected within tumoral tissue. Endometrial cancer was confirmed in 37 women (3 premenopausal). Benign hyperplasia (14 cases), secretory or proliferative endometrium (5 cases) or endometrial atrophy (2 cases) was found in the remaining women. Malignant changes were mostly noted as FIGO stage I and II (28 cases) and had a low (1 or 2) histological grade (29 cases). Median MVD’s assessed with CD105 and CD34 were 10.4 and 32.3, respectively. Median MVD assessed with CD34 was almost twice higher in women with benign and malignant endometrial changes. The study included 58 women (37 postmenopausal) with normal, hyperplastic and malignant endometrium in which preoperative transvaginal sonography was performed. Histological results of the removed endometrium were correlated with MVD assessed in “hot areas” where high densities of microvessels were detected within tumoral tissue. Endometrial cancer was confirmed in 37 women (3 premenopausal). Benign hyperplasia (14 cases), secretory or proliferative endometrium (5 cases) or endometrial atrophy (2 cases) was found in the remaining women. Malignant changes were mostly noted as FIGO stage I and II (28 cases) and had a low (1 or 2) histological grade (29 cases). Median MVD’s assessed with CD105 and CD34 were 10.4 and 32.3, respectively. Median MVD assessed with CD34 was almost twice higher in women with benign and malignant endometrial changes than in women with benign endometrium (CD34 MVD = 41.8 vs. 27.6, p=0.004). In cases of CD105 MVD significant differences between women with benign and malignant endometrial changes were also found (CD105 MVD = 11.8, vs. 6.4; p=0.00007). The menopausal status, but not the clinical stage or histological grading was significantly correlated with both CD34 MVD (p=0.02) and CD105 MVD (p=0.0003). A significant correlation was also found between CD34 and CD105 measured MVD (p=0.000001). In conclusion, transition from endometrial hyperplasia to endometrial cancer appears to be accompanied by microvessel density changes. MVD assessed with both CD34 and CD105 antibodies could be used as a potential prognostic factor.
in women with endometrial cancer. Our study showed that endoglin, by staining the proliferating microvessels could be more specific and sensitive marker for tumor neoangiogenesis than the more commonly used marker, CD34.

**Key words:** endometrial cancer, angiogenesis, microvessel density, CD34, CD105

**INTRODUCTION**

New blood vessel formation plays an important role in the metastatic process because tumor cells easily enter the circulation via microvessels (1, 2). It has been established that endothelial cells of tumor-associated neovasculature proliferate 20–2000 times more rapidly than endothelial cells of normal tissues (3). Intratumoral microvessel density (MVD) determined by staining endothelial antigens on histological sections may be used as a quantitative measure of angiogenesis. Small blood vessels as well as capillaries can be detected on immunohistochemistry with a range of specific antigens. Many studies published to date have used factor VIII-related antigen (von Willebrand factor), while others have used markers such as CD-31 and CD-34 (4, 5). Endometrial cancer is currently the most common gynecologic malignancy (3). The transition from benign proliferative endometrium to well-differentiated adenocarcinoma probably proceeds through several intermediary steps, including various types endometrial hyperplasia. Endometrial hyperplasia (EH) and endometrial cancer (EC) are frequently highly vascularised (6, 5). Angiogenesis may be studied with the use of microvessel density (MVD) assessment performed on removed tumor sections with the use of specific antibodies that stain endothelial cells. Several researches have documented the potential usefulness of MVD in the assessment of the probability of survival (7, 8, 9). We have previously demonstrated that the increased number of microvessels was related to a significantly higher clinical stage of endometrial cancer in postmenopausal women (10). Salvesen et al. have found that the 5 years survival rate for women with high and low microvessel density was 57% and 90%, respectively (11). Several recent studies suggested that CD105 might have a higher sensitivity than panendothelial markers such as factor-VIII related antigen, CD31 or CD34 in detecting malignant tumors angiogenesis (12, 13, 14). The aim of this study was to compare the usefulness of studying microvessel density in endometrial hyperplasia and endometrial cancer with the use of the expression of two endothelial cell markers.

**MATERIAL AND METHODS**

The material for this retrospective study was obtained from women who underwent surgery at the Ist Department of Gynecological Oncology and Gynecology of the Medical University in Lublin
between the years 2004 and 2006. The histological grade (G1-G3) and clinical stage of the malignant tumors and the menopausal status of the women were recorded. The study included 58 women with endometrial changes suspected on preoperative transvaginal sonography. MVD analysis was performed on paraffin wax–embedded representative tumor tissue sections fixed in 10% neutral buffered formalin. Four-micron-thick sections, cut from paraffin blocks of the uterine tissues, were dewaxed and hydrated. They were then exposed to 3% hydrogen peroxide solution. For MVD assessment two primary antibodies were used: monoclonal mouse anti-human CD34 (clone QBEND/10; DAKO, Denmark) and monoclonal mouse anti-human CD105 (clone 4G11; Novocastra Laboratories Ltd, UK). Following antigen retrieval with the citrate buffer (0.01 M, pH 6.0), two cycles of heating in a microwave oven (each of 5 min, at 750 W) were performed. Tissue sections were incubated at 37°C with the primary antibody for 1 h (dilution: anti-CD34 - 1:25; anti-CD105 - 1:100). The slides were next incubated with the anti-mouse secondary antibody conjugated with streptavidin-biotin-peroxidase complex (LSAB2/HRP kit; DAKO, Denmark), and a color reaction was developed using DAB (3′-3-diaminobenzidine tetrahydrochloride, Sigma, USA) chromogen substrate for 5min at 37°C. The sections were counterstained with Mayer’s hematoxylin. For each case, a negative control was applied by replacing the antibody by PBS or nonimmune serum. Sections of normal endometrium were used as positive controls for anti-CD34 and anti-CD105 antibodies.

Microvessel density counting was performed by an experienced pathologist who had no knowledge of the patient’s clinical data. An Olympus CX41 (Japan) microscope with digital camera image acquisition system (Olympus DP12) was used for the histological studies. Initially, the most vascularised tumor areas were selected under low power (x40 and x100) using a light microscope to identify the areas containing the greatest number of stained vessels (so called “hot spots”). MVD was assessed under 200x magnification according to a method described by Weidner et al. with the use of “count point” option of the built-in software in five different vascular “hot spots” (4). Each brown stained cell or cell cluster that was clearly separated from adjacent microvessels, tumor cells and other connective tissue elements were considered as a single countable microvessel. Vessels characterized by thick muscular walls or with lumen greater then 20µm in diameter were excluded from the count.

Statistical analysis was performed with the use of Statistica v.6.0 (Statsoft, Poland). The Mann-Whitney U-test was used for pairwise comparisons between groups and Kruskal-Wallis ANOVA on ranks for more than two groups. The correlations were examined with the calculation of Spearman rank correlation coefficient. The criterion of statistical significance applied in all calculations in which p<0.05.

RESULTS

The mean age of the studied women was 62 years (SD ±10.1 yrs) and 37 of them were classified as postmenopausal. Endometrial cancer was found in 37 women (3 premenopausal). Benign hyperplasia (14 cases), secretory or proliferative endometrium (5 cases) or endometrial atrophy (2 cases) was found in the remaining women. Malignant changes were mostly in FIGO stage I and II (28 cases) and had a low (1 or 2) histological grade (29 cases). Vascular density was quite heterogeneous in any given tissue section of the various histologic types, some areas being vascular and other areas remaining relatively avascular. Small capillaries of less than 10µm in maximum diameter were mainly stained
with CD105. Antibodies to CD34 were attached to small and large microvessels with a similar intensity. Median MVD’s assessed with CD-105 and CD-34 were 10.4 (range: 2.3-36.8) and 32.3 (range: 21.1-41.8), respectively. Median MVD assessed with CD-34 was almost twice as high in women with endometrial cancer than in women with a benign endometrium (medians CD-34 MVD = 41.8 vs. 27.6, Z=-2.85; p=0.004). In case of CD-105 MVD, significant differences between women with benign and malignant endometrial changes were also found (median CD-105 MVD = 11.8, range: 6.8-36.8 vs. 6.4; range: 2.4-11.2; Z=-3.96; p=0.00007). The menopausal status, but not the clinical stage or the histological grading was significantly correlated with both CD-34 MVD (p=0.02) and CD-105 MVD (p=0.0003). A significant correlation was found between CD-34 and CD-105 measured MVD (Spearman’s R=0.60, p=0.000001).

DISCUSSION

The assessment of angiogenic factors in cancer patients may have important clinical implications in many malignant tumors. Several researchers have measured angiogenesis by correlating the microvessel counts with tumor aggressiveness (15, 16). The degree of angiogenesis could allow for the identification of patients at high risk of recurrence who may benefit from aggressive surgical procedures and/or neoadjuvant therapy (13). A recent report by Salvesen et al. demonstrated that angiogenesis in endometrial carcinoma correlates with tumor grade and the depth of the invasion (17). In our study, we analyzed the expression of CD105 in newly formed blood microvessels compared with that of CD34, which is regarded as one of the “classical” markers for the assessment of MVD. Our results indicate that antibodies to CD34 antigens is highly sensitive to all endometrial cancer microvessels, but it may have a poor specificity for measuring angiogenesis, if based on the immunohistochemical staining technique alone. In several reported studies the MVD counts using anti-CD105 antibody significantly correlated with the overall and disease-free survival, whereas no such correlation was seen using the pan-endothelial marker such as CD34 (7, 12, 18). In our series, the microvessel density, indicated by the expression of CD34 and CD105 antibodies, varied according to the tumor type. The median number of microvessels found in serial sections of EC with CD105 was significantly lower than for CD34, which indicates that endoglin might be more useful for the identification of newly formed, neoplastic microvessels. If angiogenesis factors and MVD assessment could predict distant metastasis in women with endometrial cancer, the adjuvant therapy could be changed. Transition from endometrial hyperplasia to endometrial cancer appears to be accompanied by microvessel density changes. Previous research and our present study indicate that MVD assessed with both CD-34 and CD-105 antibodies could be an important additional factor to endometrial cancer’s histological and clinical features.
The potential usefulness of various antibodies used for the precise assessment of microvessel density and blood flow around normal and hyperplastic endometrium remains controversial (15, 17). None of the widely used panendothelial markers such as: CD31, CD34 or vWF–VIII is specific enough to indicate all proliferating microvessels, and because of this new attempts to quantify neoangiogenesis in malignant tumors are undertaken. In the last few years endoglin (CD105) was suggested to be a new potential indicator of active angiogenesis. Antibodies to CD105 have a higher specificity than panendothelial markers in the identification of new microcapillaries, the former being specific mainly for larger capillaries and host organ blood vessels adapted by the developing malignancy (14).

Our present study indicated that antibodies to CD34 identified both small (i.e. less than 20 µm) and large (i.e. more than 50 µm) capillaries with equal intensity of staining. We found that the median of CD34 stained microvessel density was nearly 3 times higher than MVD for CD105 stained microvessels. Both MVD’s were highly correlated with each other. This significant difference in the average number of microvessels stained by both antibodies indicates that endoglin could serve as a marker of proliferating endometrial vessels with no expression in mature microvessels.

In a similar study that compared CD31 and CD105 in endometrial cancer, Salvesen et al. observed more intensive staining of microvessels for endoglin when compared to CD31. They have also found that in normal endometrial tissue, only approximately 20% of all microvessels could be identified with CD105 staining (17). The authors have concluded that the use of this marker could reduce the number of false positive staining spots, an event that is frequently encountered when panendothelial antibodies are used to assess tumor MVD.

Our results presented above indicate that microvessel density assessed with CD34 or CD105 antibodies was almost two times higher in malignant endometrial changes than MVD in endometrial hyperplasia. These observations are in line with the data presented by Saad et al. who found that in women with endometrial cancer, the average numbers of microvessels per high power field (HPF) stained with CD31 and CD105 were 30,8/HPF i 13,3/HPF, respectively (18). Both markers were highly correlated with each other, additionally a significant correlation of CD31-MVD with the depth of invasion and histological grading of endometrial cancer was observed. Interestingly, a significant correlation with lymph node metastases and lymphovascular space involvement was found only for CD105 assessed microvessel density. Our study indicated that an important factor influencing microvessel density in endometrial lesions was the menopausal status of the patient. Premenopausal women had a significantly lower CD34 and CD105 assessed MVD than postmenpausal patients. This observation could be explained by a high prevalence of endometrial cancer in our studied group, where the mean age of affected women was approximately 60 years with only 3 patients in this group being premenopausal. The histological grading of endometrial cancer had no significant influence on microvessel density.
identified with both markers used in this study. These observations are in line with the recent study published by Erdem et al. (19) who compared survival rates with MVD in endometrial cancer samples. The authors noted that women with high CD-105 MVD had a significantly worse prognosis than patients with low MVD assessed with this marker. Interestingly, in this study no such correlation was found for CD34 assessed MVD.

Ozalp et al. (20) have found that microvessel density in endometrial cancer was significantly higher than in the control group of normal proliferative or secretory endometrium. These authors have found that the microvessel density was highly correlated with the clinical stage, myometrial invasion, lymph node involvement and ascites. In summary MVD assessed with both CD-34 and CD-105 antibodies could be used as a potential prognostic factor in women with endometrial cancer. Endoglin could be used as a more specific and sensitive marker of tumor neoangiogenesis than commonly used endothelial markers.

Conflict of interest statement: None declared.

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