

# Vascular markers CD31, CD34, actin, VEGFB, and VEGFR2, are prognostic markers for malignant development in benign endometrial polyps

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## ABSTRACT

**Intention of the study:** The prevalence of endometrial polyps has been demonstrated in between 10% and 35% of all women, but knowledge regarding malignant potential within polyps is limited. Even though premalignant and malignant changes have been reported in up to 24% of all cases, no objective tissue markers have ever been developed for routine diagnostics to select high risk cases. As vascular changes and activation of endometrial angiogenesis has been demonstrated in former studies, our main objective was to evaluate different members of the angiogenic pathway as potential risk factors for cancer development. **Patients and methods:** Formalin-fixed, paraffin-embedded tissue from 15 women with benign endometrial polyps, and 16 women diagnosed with endometrial cancer were included. Immunohistochemical investigation with antibodies against VEGF, VEGF-B, VEGFR2, VEGFR3, CD31, CD34, actin, and factor-VIII was performed, followed by evaluation of staining intensity of microvessels, evaluation of H-score in glands (cell membrane, cytoplasm) and stroma, and measurement of micro vessel density. **Results:** Expression of CD31 in microvessels was significantly stronger in cancers compared to endometrial polyps ( $P = 0.006$  for arterioles,  $P = 0.038$ , for venules, and  $P = 0.002$  for capillaries, respectively), whereas, a reverse change was shown for CD34. Expression of actin in capillary walls was also significantly increased in cancers compared to polyps ( $P = 0.002$ ). No significant difference was found for staining intensity in microvessels (arterioles, venules or capillaries) in endometrial benign polyps compared with endometrial cancers for VEGF, VEGFB, VEGFR2, VEGFR3, or Factor VIII. Also no difference in H-score values between benign polyps

and endometrial cancers could be detected in glandular epithelium, in epithelial cell membrane or in stroma for VEGFR3, CD31 or Factor VIII. **Conclusions:** The present study strongly indicates that activation of angiogenesis differs in benign endometrial polyps and endometrial cancers. Thus, immunohistochemical expression of specific angiogenic markers may be of great importance as prognostic factors in the routine diagnostics of this lesion. The ratio between stromal expression of CD34 and actin might be of particular interest to select polyps with increased malignant potential.

**Keywords:** Endometrial Polyps; Endometrial Cancer; Vascular Markers; Prognosis

## 1. INTRODUCTION

Although endometrial polyps are frequently occurring structures, routine diagnostic and routine therapy based on objective and profound knowledge of malignant potential is limited. The prevalence of endometrial polyps shows great differences in comparable studies varying between 10% in one population and up to 35% in others [1-6]. Polyps exceeding a certain size are easily visualized by transvaginal ultrasound, sonography or hysteroscopy, but the large variation in reported prevalence may reflect the difficulties in confirming the histological diagnosis. Thus, polyps removed by curettage are often fragmented and therefore overlooked or neglected by the pathologist.

Malignant transformation in endometrial polyps is known to occur in only a minority of all lesions and even if results from different studies may be discordant, most endometrial polyps are known to be benign lesions [1]. In one former study of polyps from women presenting

with abnormal uterine bleeding 23.8% showed pre-malignant changes (complex and atypical hyperplasia) and 1.5% had endometrial cancer [1]. In another report 11.3% of the studied polyps had hyperplasia and 3.2% were malignant [7]. However, malignancy in endometrial polyps associated with tamoxifen use has been reported to be considerably more frequent [3,8].

Thus, endometrial hyperplasia and endometrial polyps may be considered as risk factors for endometrial carcinoma and some evidence exist that polyps may be indicators of malignant transformation [9]. In histopathological evaluation of premalignant endometrial lesions it is widely accepted that development of endometrial carcinoma may occur through a spectre of hyperplastic lesions, varying from simple hyperplasia to complex atypical hyperplasia.

Although never proved, similar changes have been expected to occur in polyps. Malignant change in endometrial hyperplasia is reported to vary from 2% up to more than 50% in different materials [10-13]. Whether these criteria, which have been seriously criticized for low reproducibility, are predictive for malignant development within endometrial polyps has never been seriously confirmed.

So far, the knowledge regarding risk factors of malignant development in endometrial polyps is limited and no objective reliable clinical or biological markers exist for the prediction of progression to endometrial carcinoma. In the search for objective, prognostic markers in endometrial polyps Lieng and co-workers recently found that pulse wave Doppler assessment of downstream resistance in arterioles was significantly different in endometrial polyps compared to endometrial cancers [14]. In the endometrium from healthy, fertile women, expression of angiogenic markers like VEGF-A, -B, and -C, and VEGF receptors 1-3 in endometrial blood vessels indicate a highly structured involvement of angiogenesis [15].

Increasing levels of angiogenesis has been reported in endometrial hyperplasia and cancer compared to normal

proliferative endometrium [16]. Previous studies of endometrial cancers additionally have demonstrated an association between prognosis and level of angiogenesis [17,18]. Thus, more exact diagnostics in daily routine for the selection of polyps with increased malignant potential would be essential. Against this background, we decided to investigate whether immunohistochemical expression of angiogenic markers were different in endometrial carcinoma compared to benign endometrial polyps.

## 2. PATIENTS AND METHODS

### 2.1. Patients

This prospective study included 15 consecutive women with benign endometrial polyps diagnosed by saline contrast sonohysterography (SCSH), and 16 consecutive women with endometrial cancer. The women with endometrial polyps were referred to the Department of Gynecology at Ullevaal University hospital following incidental sonographic findings suggestive of endometrial polyp. The women with endometrial carcinoma were referred after histologically verified malignancy in endometrial biopsy. The study protocol was approved by the local research ethics committee, and written and oral informed consent was obtained from all study participants.

### 2.2. Immunohistochemistry

Biopsies were routinely fixed in 4% buffered formaldehyde and embedded in paraffin before 5 µm histological sections were made, stained with eosin and haematoxylin. Histological investigation of the routine specimens was performed by a gynaecologic pathologist (BR). Immunohistochemistry was performed according to customer's advice and the different procedures described in **Tables 1** and **2**. The eight different investigated primary antibodies were against VEGF, VEGF-B, VEGFR2, VEGFR3, CD31, CD34, actin, and factor VIII.

**Table 1.** Procedures performed for the antibodies VEGF, VEGF-B, VEGFR-2 and VEGFR-3. Immunohistochemistry was performed according to customer's advice.

Procedure/Antibody tested	VEGF	VEGFB	VEGFR2	VEGFR3
Primary antibody	Cat nr: RB-9031 Rabbit polyclonal	Cat nr: MAB751 Mouse monoclonal	Cat nr: RB-9239-P1 Rabbit polyclonal	Cat nr: RB-9255-P Rabbit polyclona
Negative control Diluent only	Multitissue titration block	Multitissue titration block	Multitissue titration block	Multi tissue titration block
Positive control	Multitissue titration block	Multitissue titration block	Multitissue titration block	Multi tissue titration block
Company/purchaser	Lab Vision, California, USA	R & D Systems, Minnesota, USA	Lab Vision, California, USA	Lab Vision, California, USA
Pre-treatment procedure Staining	Boil in TrisEDTA-buffer standard Dako Autostainer	Boil in TrisEDTA-buffer, standard Dako Autostainer	Boil in TrisEDTA-buffer, standard Dako Auto-stainer	Boil in TrisEDTA-buffer, standard Dako Autostainer
Titre	1/150	1/20	1/100	1/300

**Table 2.** Procedures performed for the antibodies CD31, CD34, actin and Factor VIII. Immunohistochemistry was performed according to customer's advice.

Procedure/Antibody tested	CD31	CD34	Actin	Factor VIII
Primary antibody	Cat nr: M 0823 Mouse monoclonal	Cat nr: 790-2927 Mouse monoclonal	Cat nr: 760-2502 Mouse monoclonal	Cat nr: 760-2642 Rabbit polyclonal
Negative control Diluent only	Multitissue titration block	Multitissue titration block	Multitissue titration block	Multitissue titration block
Positive control	Multitissue titration block	Multitissue titration block	Multitissue titration block	Multitissue titration block
Company/purchaser	Dako Cytomation, Denmark	Ventana Medical Systems, Arizona,US	Ventana Medical Systems, Arizona, US	Ventana Medical Systems, Arizona,US
Pre-treatment: procedure Staining	Boil inTRS ph 6,1 Dako Autostainer	Standard Ventana program Ventana Autostainer	Standard Ventana program Ventana Autostainer	Standard Ventana program Ventana Autostainer
Titre	1/40	Ready to use lot	Ready to use lot	Ready to use lot

### 2.3. Evaluation of Immunohistochemical Expression in Epithelium, Epithelial Cell Membrane, and Stroma (H-Score)

The results of immunostaining in epithelial tissue (epithelium in endometrial glands in polyps and malignant epithelium in cancers), epithelial cell membranes, epithelial cell cytoplasm and cytoplasm in stroma were examined semi-quantitatively by using an immunohistochemical histological score (H-score) which incorporates both the intensity and the distribution of specific staining. The H-score has been previously formulated as

$$HS = \sum (P_i \times i) / 100,$$

where  $P_i$  denotes the percentage of stained cells and  $i$  denotes the intensity of the staining ranging from 1 - 3 [19]. Epithelial tissue (epithelial cell membranes, epithelial cell cytoplasm) and cytoplasm in stroma were classified separately. A specific area within each specimen of polyps and carcinomas (hot spots) was defined for investigation by a gynecological pathologist (AØ). For each of the eight different antibodies used for immunohistochemical staining, this specific area was investigated for each case with reference to epithelium, cell membranes and stroma. Both staining intensity and number of positive cells were counted. Less than 10% positive cells were considered as negative and characterized as 0, 10% - 30% positive cells were assessed as 1, 31% - 70% positive cells as 2, and 71% - 100% positive cells as 3. In evaluating the staining intensity, a score of zero indicated an absence of staining. Scores of 1, 2 and 3 indicated weak, moderate and strong immuno-reactivity, respectively. The H-score evaluation was performed visually as a consensus between a trained gynecologic pathologist (AO) and a PhD student (BH). Both investigators were blinded to the two patient groups. Internal protein control was performed regularly for each staining in cancers as

well as in polyps.

### 2.4. Evaluation of Immunohistochemical Staining Intensity in Microvessels

A semi quantitative evaluation of staining intensity in microvessels: arterioles (vessels with defined muscular wall), capillaries (smallest vessels taking no more than a couple of erythrocytes), and venules (larger diameter than capillaries lacking muscular wall) was performed by light microscopy as a consensus between a trained gynecologic pathologist (AO) and a PhD student (BH). Both investigators were blinded to the two patient groups. Light staining was evaluated as score = 1, moderate staining as score = 2 and strong staining as score = 3. Finally, the intensity score values were compared for endometrial polyps and for cancers for each specific immunohistochemical antibody.

### 2.5. Microvessel Density Measurement

Microvessel density measurement was performed after CD 34 staining of the specimens. A modified Chalkley count method was utilized to measure microvessel density [20]. Within stroma of polyps and cancers, three areas of most intense neovascularization (vascular hot-spots) were selected and marked for each specimen. Using the Q Prodit image analysing system (version 6.1; Leica, Cambridge, UK) with 30 micrometer grid lines the number of microvessels were counted within each separate hot-spots (magnification 40×) and results were presented as mean of the three counts.

### 2.6. Statistics

Mann-Whitney two tailed unpaired test, GraphPad InStat version 3.06, GraphPad Software, San Diego California USA were employed for all statistical evaluations in the

present study for comparison of patient groups using. P-levels  $\leq 0.05$  were considered significant of the tree defined areas. Any stained endothelial cell or cell cluster separated from adjacent microvessel

### 3. RESULTS

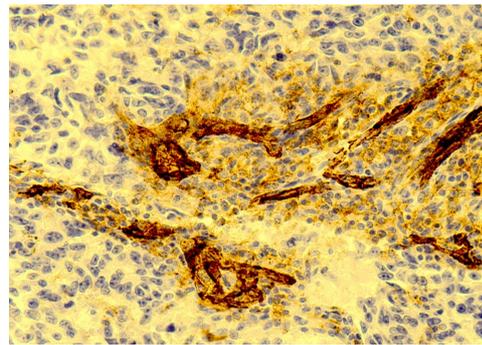
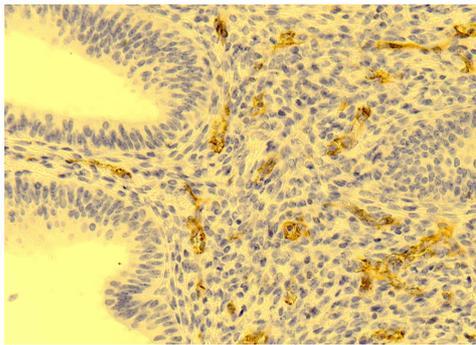
#### 3.1. Patients

The mean age of the patients with polyps was  $58.0 \pm 8.1$  and for the cancer patients  $63.1 \pm 10.3$ . Body mass index for the patients with polyps and for the cancer patients was  $27.5 \pm 5.8$  and  $28.8 \pm 4.8$  0.5, respectively. Endometrial thickness was described in mm as  $15.1 \pm 8.2$  for the patients with polyps and  $20.6 \pm 10.4$  for the cancer patients. Volume of polyp and tumour volume was estimated in ( $\text{cm}^3$ ) to be  $6.4 \pm 8.3$  and  $10.9 \pm 6.9$  for polyps and cancers, respectively. Of all the included patients only five were premenopausal. Among the patients with

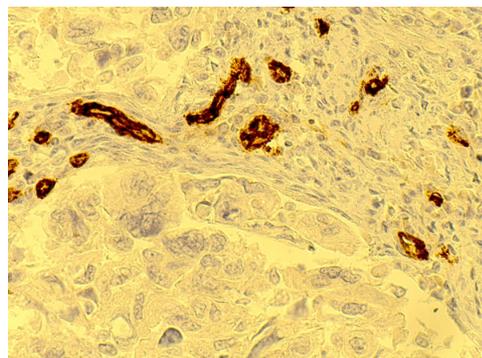
carcinomas, 14 were diagnosed as endometrioid carcinomas (all Figo stage 1 or Figo stage 2), one as seropapillary carcinoma (Figo stage 1), and one as clear-cell carcinoma (Figo stage 3). All the included polyps were diagnosed by the pathologist to be without atypia. All patients with cancers were treated according to the national routines.

#### 3.2. Results of Staining Intensity in Microvessels

Evaluation of immunohistochemical expression demonstrated that expression of CD31 was significantly stronger in microvessels (arterioles, venules, and capillaries) in cancers compared to endometrial polyps (**Figure 1**, and **Table 3(a)**). Reversely, for CD34, the intensity of immunohistochemical expression was reduced in cancers in all three types of microvessels compared to endometrial polyps; however, the reduction was significant only in venules (**Figure 2** and **Table 3(b)**). Also for actin the



**Figure 1.** Left panel: The micro photo from an endometrial polyp shows expression of CD31 in microvessels (arterioles, venules and capillaries) with endothelial cells staining light brown. The microvessels are surrounded by stromal cells which are negatively stained (blue). In the left part of the picture an endometrial gland is also demonstrated to be negative for CD31 (20 $\times$  magnification). Right panel: The micro photo from endometrial cancer shows expression of CD31 in endothelium of microvessels (arterioles, venules and capillaries) staining dark brown. The microvessels are surrounded by stromal cells which do express some CD31 showing light brown colour within cellular cytoplasm (20 $\times$  magnification).



**Figure 2.** Left panel: The micro photo from an endometrial polyp stained with CD34 showing negatively stained glands surrounded by dark brown stromal cells expressing CD34. Dark brown stained microvessels are also localized within the stroma (40 $\times$  magnification). Right panel: In contrast the micro photo of an endometrial cancer shows negatively stained stromal tissue with strongly positive microvessels (bridge of stromal tissue in the centre). Endometrial cancerous tissue surrounding stroma seen on both sides is also negatively stained (blue) (20 $\times$  magnification).

staining intensity was increased in cancers compared to intensity observed in endometrial polyps, however, statistical difference was observed exclusively in capillaries (**Figure 3** and **Table 3(c)**). No significant difference was found for staining intensity in microvessels (arterioles, venyles or capillaries) in endometrial benign polyps compared with and endometrial cancers for VEGF, VEGFB, VEGFR2, VEGFR3, or Factor VIII (data not shown).

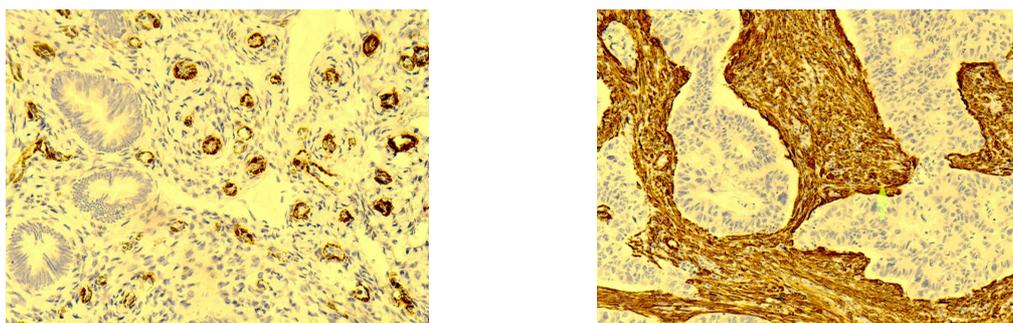
### 3.3. Results of H-Score in Epithelium of Glands (Cytoplasm and Epithelial Cell Membrane) and Stroma (H-Score)

VEGF was expressed with high H-score in cytoplasm of epithelial cells as well as in stroma in both polyps and cancers but no significant difference was found (**Table 4(a)**). For VEGFB a significant reduction in H-score in epithelial cell membrane as well as in stroma was observed for cancers compared to endometrial polyps, whereas, in cytoplasm of the same cells no difference

was detected (**Table 4(b)**). An increased H-score level in cytoplasm of glandular tissue in cancers was observed for VEGFR2 compared to polyps, but no difference in H-score in stromal tissue could be observed (**Table 4(c)**). H-score values for CD34 in stroma was significantly reduced in cancers compared to endometrial polyps, whereas, stromal actin levels increased (**Figures 2** and **3**, **Tables 4(d)** and **(e)**). No difference in H-score values between benign polyps and endometrial cancers could be detected in glandular epithelium, in epithelial cell membrane or in stroma for VEGFR3, CD31 or Factor VIII.

### 3.4. Result of Microvessel Density Measurements

Image analyses of microvessel density in polyps and in cancers showed that the mean value of micro vessel density was 7.400 ( $\pm 2.02$ ) for polyps and 6.071 ( $\pm 2.24$ ) for cancer, respectively. The results from the two groups were not significantly different ( $P = 0.07$ ).



**Figure 3.** Left panel: This photo shows an endometrial polyp with benign looking glands surrounded by stroma. Neither stroma nor do glandular tissue do express actin, however, microvessel staining is shown in capillaries which are dark brown (20 $\times$  magnification). Right panel: This micro photo shows endometrial cancer tissue stained with actin. The negatively stained malignant glandular tissue is surrounded by stroma stained dark brown demonstrating strong expression of actin (40 $\times$  magnification).

**Table 3.** Differences of immunohistochemical score for expression of CD31, CD34 and actin in microvessels in polyps and cancers.

(a) Expression of CD31			
Localization of staining	Benign polyps Mean $\pm$ SD (N = 15)	Cancer Mean $\pm$ SD (N = 16)	p-value
Arterioles	31.3 $\pm$ 30.2	58.8 $\pm$ 20.6	0.006
Venyles	44.7 $\pm$ 22.3	60.6 $\pm$ 18.4	0.038
Capillaries	48.7 $\pm$ 23.6	71.9 $\pm$ 13.3	0.002
(b) Expression of CD34			
Localization of staining	Benign polyps Mean $\pm$ SD (N = 15)	Cancer Mean $\pm$ SD (N = 16)	p-value
Arterioles	80.0 $\pm$ 8.5	68.3 $\pm$ 24.0	0.087
Venyles	80.0 $\pm$ 8.5	65.0 $\pm$ 25.1	0.037
Capillaries	79.3 $\pm$ 8.8	69.0 $\pm$ 23.9	0.128
(c) Expression of actin			
Localization of staining	Benign polyps Mean $\pm$ SD (N = 15)	Cancer Mean $\pm$ SD (N = 16)	p-value
Arterioles	70.7 $\pm$ 8.0	72.5 $\pm$ 6.8	0.49
Venyles	70.7 $\pm$ 8.0	72.5 $\pm$ 6.8	0.34
Capillaries	21.3 $\pm$ 20.0	73.8 $\pm$ 7.2	<0.0001

**Table 4.** H-score for VEGF, VEGFB, VEGFR2, CD 34 and actin in glands and stroma in polyps and cancers, respectively.

(a) Expression of VEGF in cytoplasm. Cell membrane was unstained in polyps as well as in cancers.			
Localization of staining	Benign polyps Mean $\pm$ SD (N = 15)	Cancer Mean $\pm$ SD (N = 16)	p-value
Glandular cells	184.7 $\pm$ 66.8	132.5 $\pm$ 80.7	0.167
Stromal cells	20.6 $\pm$ 33.4	25.0 $\pm$ 36.3	0.921
(b) Expression of VEGFB in cytoplasm (CP) and cell membrane (CM) of glandular cells and cytoplasm (CP) of stromal cells. Cell membrane was unstained in polyps.			
Localization of staining	Benign polyps Mean $\pm$ SD (N = 15)	Cancer Mean $\pm$ SD (N = 16)	p-value
Glands/CP	53.6 $\pm$ 41.8	63.1 $\pm$ 48.0	0.525
Glands/CM	0.93 $\pm$ 0.47	0.13 $\pm$ 0.34	0.0003
Stroma/CP	32.1 $\pm$ 30.7	2.5 $\pm$ 10.0	0.012
(c) Expression of VEGFR2 in cytoplasm (CP) and cell membrane (CM) of glandular cells and cytoplasm (CP) of stromal cells. Cell membrane was unstained in polyps.			
Localization of staining	Benign polyps Mean $\pm$ SD (N = 15)	Cancer Mean $\pm$ SD (N = 16)	p-value
Glands/CP	2.3 $\pm$ 5.6	25.6 $\pm$ 31.4	0.0137
Glands/CM	0.47 $\pm$ 0.52	0.13 $\pm$ 0.34	0.109
Stroma/CP	2.7 $\pm$ 7.0	11.9 $\pm$ 36.4	0.625
(d) Expression of CD34 in cytoplasm. Cell membrane was unstained in polyps as well as in cancers.			
Localization of staining	Benign polyps Mean $\pm$ SD (N = 15)	Cancer Mean $\pm$ SD (N = 16)	p-value
Glandular cells	0	0	-
Stromal cells	93.3 $\pm$ 90.1	12.5 $\pm$ 27.2	0.0122
(e) Expression of actin in cytoplasm. Cell membrane was unstained in polyps as well as in cancers.			
Localization of staining	Benign polyps Mean $\pm$ SD (N = 15)	Cancer Mean $\pm$ SD (N = 16)	p-value
Glandular cells	0	0	-
Stromal cells	54.3 $\pm$ 83.1	217 $\pm$ 48.4	0.0002

#### 4. DISCUSSION

As far as we know, this is the first report studying angiogenic markers in histopathological material comparing benign polyps and cancers. As endometrial cancer is now the most frequent gynaecological malignancy in the Western world and the incidence is constantly increasing, potential biological markers for cancer development to select high risk cases for surgical treatment would be of importance in daily routine. In former studies other risk indicators of malignant development in polyps like age, menopausal status, polyp diameter, and hypertension have been evaluated [1,21-23]. Thus, in a recent study polyp diameter exceeding 18 mm was significantly associated to an abnormal histology [22]. Also Rahimi and co-workers concluded that postmenopausal women with larger polyps had a 3.6-fold higher likelihood of atypia [23]. However, such indicators have limited value as they do not exclude malignancy in smaller lesion and more exact diagnostic guidelines are important to determine optimal and individualized therapy.

Among the most remarkable result in the current study of angiogenic markers was the reduction in CD34 expression in stromal tissue coinciding with increased expression of stromal actin in cancers compared to polyps. Correspondingly, in a study of non invasive cervical cancer a dense network of CD34 positive stromal fibrocytes was observed, whereas, the stroma of invasive carcinoma was virtually free of this cell population [24]. Early stromal invasive squamous carcinoma was also characterized by a focal loss of expression of CD34 fibrocytes. In normal cervical stroma actin positive myofibroblasts were not seen [24]. Similar results were confirmed in another study from premalignant and malignant disease of the uterine cervix demonstrating that the disappearance of CD34-positive stromal cells and appearance of actin positive stromal myofibroblasts were associated with malignant transformation [25]. Also in studies from breast pathology it has been shown that reduction in CD34 expression and concurrent increase in actin may be considered as helpful tools in distinguishing benign breast lesions (e.g., sclerosing adenosis) from inva-

sive breast cancer [26].

Increased single expression of actin has also been considered in diagnostics of breast cancer and in a recent study from Yamashita and co-workers the authors conclude that stromal myofibroblasts stained immunohistochemically by alpha smooth muscle actin represent an important prognostic factor for invasive growth that may be translated into a poor clinical prognosis for patients with invasive breast cancer [27].

Other studies have also asserted that the stromal reaction in different carcinomas may be of major importance and CD34-positive stromal cells may play a significant role in the host response to tissue damage showing immune-mediated host defence against invading tumour cells [24,25]. In a recent study Nakayama and co-workers described that loss of CD34 expression in stroma was associated with invasive carcinoma and in part, accompanied by a gain of actin positive myofibroblasts in the stroma of colorectal cancer cells [28]. Most of the stromal cells in the normal colorectal submucosa, muscularis propria, subserosa, and perirectal tissue were positive for CD34. In contrast, the peritumoral inflammatory tissue and the tumor stroma had no CD34 positive stromal cells [28]. Although further investigations are essential, expression of CD34 and actin in stromal tissue within endometrial polyps with different malignant potential may become important future markers for evaluation of malignant potential and cancer development.

Another remarkable finding in the current study was the significantly increased expression of the pan-endothelial marker CD31 in arterioles, capillaries and venules in endometrial cancers compared to polyps. Former studies investigating the significance of CD31 expression as a prognostic marker in endometrial polyps have never been performed. In a recent retrospective immunohistochemical study evaluating expression of CD31 and CD105 (endoglin) in pancreatic cancers with different outcome, the authors concluded that angiogenesis-associated molecules like CD31 and CD105 might be useful tools as prognostic markers in pancreatic cancers [29]. On the other hand, for the second endothelial marker, CD34, the expression of CD34 was shown to be reduced in microvessels (venules) in cancers compared to polyps in the present study.

In the present study VEGFR2 expression showed an increased H-score in cytoplasm of glandular tissue in the endometrial cancers compared to polyps. Although no significant difference between the two groups was asserted, VEGF was substantially expressed in both groups with high H-score levels. In a recent study Koukourakis and co-workers demonstrated that serum levels of VEGF were increased in patients with breast and gynecological malignancies but also in certain benign conditions, including cases of fibrocystic disease of the breast and in

simple endometrial hyperplasia [23,30]. They also found that expression of phosphorylated VEGFR2/KDR receptors was higher in breast, endometrial, and ovarian cancer in patients with high VEGF serum levels reaching a level of statistical significance when all malignancies were combined. Further, a stimulatory effect of serum VEGF on the VEGF pathway contributing to tumour progression was indicated [23,30]. In our study serum levels of VEGF was never estimated although epithelial expression was outstanding with high H-score levels in both groups. Thus, increased H-score for VEGFR2 in malignant epithelium, as demonstrated in our results, may indicate increased stimulatory effects of VEGF in pathological angiogenesis and VEGFR2 might work as a marker for malignant transformation independent of VEGF [31].

Measurement of micro vessel density was also performed in areas of most active neo-vascularisation in polyps as well as in cancers. However, no significant difference between the two groups could be detected. No other studies exist in the literature investigating micro vessel density as prognostic factor for malignant development in endometrial polyps.

The present study strongly indicates that angiogenesis is different in benign endometrial polyps and in endometrial cancers. Thus, immunohistochemical expression of specific angiogenic markers may be of great importance as prognostic factors in the routine diagnostics of this lesion. Particularly the ratio between stromal expression of CD34 and actin might be of interest to select polyps with increased malignant potential. To obtain more profound knowledge within this area larger series of endometrial polyps with long-term follow is required. Such studies are in progress.

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