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Preoperative Assessment of Endometrial Carcinoma

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ACADEMIC DISSERTATION
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for public discussion in the Small Auditorium of Building M,
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To Ella, Eino and Ani

1. Abstract

The cornerstone of the treatment of endometrial carcinoma is surgery, including hysterectomy and bilateral salpingo-oophorectomy. If the risk for metastases is estimated to be increased, a pelvic and para-aortic lymphadenectomy is also warranted. Preoperative risk assessment is based on the histopathologic analysis of the diagnostic endometrial biopsy or curettage specimen, and the determination of myometrial invasion of the tumor using imaging methods. Deep myometrial invasion (>50% of the myometrial thickness) has been found to be an independent prognostic factor for metastases in endometrial carcinoma. As the extent of the operation is dependent on the results of the preoperative assessment, a good diagnostic performance of the used methods is fundamental.

One hundred consecutive patients presenting with endometrial carcinoma and scheduled for an operation at Tampere University Hospital from 2007 through 2009 were enrolled in this prospective observational study. The primary objective was to evaluate the feasibility of three-dimensional power Doppler angiography (3DPDA) in the preoperative assessment of deep myometrial invasion. All patients were examined preoperatively, and the results were correlated with the final histopathological report of the surgical specimen. The endometrial volume with endometrial and myometrial vascular indices VI (vascularization index), FI (flow index) and VFI (vascularization flow index) were calculated by 3DPDA. According to multivariate regression analysis, endometrial volume and endometrial FI were the independent predictors of deep myometrial invasion (OR, 1.109; 95% CI, 1.011–1.215 and OR, 1.061; 95% CI, 1.023–1.099. p=0.028 and 0.001, respectively). The distance between the ultrasound probe and the target tissue was found to be a notable confounding factor, which must be acknowledged when evaluating the results.

The second objective was to compare the performance of 3D sonography and magnetic resonance imaging (MRI) in a subset of 20 patients. MRI was found to be more sensitive (91.7%) in detecting deep invasion. However, 3D sonography was more specific (87.5%). A combination of the assessed methods was found to have the best or 80.0% accuracy.

The third aim of the study was to evaluate the performance of two ovarian cancer biomarkers, CA125 and HE4, in the preoperative evaluation of endometrial carcinoma. A combination of the markers, a risk score, was found to better predict

advanced stage than either of the markers alone with a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 57.1%, 98.7%, 88.9%, 92.6%, and 92.2%, respectively. Patient's BMI was found to have an influence on the level of HE4. This confounding factor must be taken into account when using HE4 measurement in clinical practice.

The last objective of the study was to incorporate angiogenic markers in the preoperative assessment. Preoperative serum concentrations of endoglin, vascular endothelial growth factor VEGF and its soluble receptor sFLT-1 were measured and correlated with the histopathological features of the tumors. Immunohistochemistry was used to assess the tumoral expression of endoglin, VEGF, and its cell surface receptors VEGFR1 and VEGFR2. Serum concentration of VEGF was found to correlate with the presence of metastases. The tumor microvessel density, assessed by immunohistochemistry, was associated with the degree of vascularization determined by 3DPDA.

The results of the present study suggest that endometrial volume measurement and endometrial blood flow assessment by 3DPDA may facilitate the preoperative workup of patients with endometrial carcinoma. In addition, the measurement of serum concentrations of CA125 and HE4 with risk score calculation may further assist identifying the patients with an elevated risk for metastases.

2. Tiivistelmä

Endometriumkarsinooman hoito on leikkaus, jossa poistetaan kohtu sekä munasarjat ja munanjohtimet. Osalle potilaista, joilla ennen leikkausta tehtyjen tutkimusten perusteella ajatellaan olevan kohonnut riski levinneeseen tautiin, tulisi tehdä myös lantion ja para-aortaalialueen imusolmukkeiden poisto. Tämän vuoksi päätös leikkauslaajuudesta tulisi perustua riittävän tarkkaan levinneisyyden riskin arvioon. Tavallisesti tämä riskin arviointi perustuu kuvantamismenetelmillä saatuun tietoon kasvaimen invaasiosta kohtulihakseen sekä patologin arvioon ennen leikkausta otetusta endometriumnäytteestä. Kasvaimen syvän invaasion (yli 50 % kohtulihaksen paksuudesta) on todettu lisäävän etäpesäkkeiden riskiä merkitsevästi.

Tähän tutkimukseen valittiin sata Tampereen yliopistollisessa sairaalassa vuosina 2007–2009 endometriumkarsinooman vuoksi leikkaushoidossa ollutta potilasta. Tutkimuksen päätavoite oli arvioida kolmiulotteisen energiadopplerkuvantamisen soveltuvuutta kasvaimen kohtuinvaasion määrittämiseen. Tutkimukseen valituille potilaille tehtiin ennen leikkausta kolmiulotteinen energiadopplertutkimus, ja tästä tutkimuksesta saatuja tuloksia verrattiin leikkausnäytteestä arvioituun invaasioon. Menetelmällä arvioitiin endometriumin tilavuus sekä laskettiin endometriumin ja kohtulihaksen verenkiertoa kuvaavat vaskulariteetti-indeksit. Endometriumin tilavuus ja endometriumilta mitattu kasvaimen verenkierron intensiteettiä kuvaava indeksi FI olivat monimuuttuja-analyysin perusteella itsenäisiä kasvaimen syvää invaasiota ennustavia tekijöitä (kerroinsuhde 1,109; 95 % luottamusväli 1,011–1,215; p=0,028 ja kerroinsuhde 1,061; 95 % luottamusväli 1,023–1,099; p=0,001). Etäisyys ultraäänianturin ja kohdealueen välillä vaikutti merkitsevästi saatuihin verenkiertoa kuvaaviin indekseihin. Tämän tuloksiin vaikuttavan tekijän huomioonottaminen on tärkeää, joskin sen kumoaminen on nykymenetelmin vaikeaa.

Toinen tavoite oli verrata kolmiulotteista ultraäänitutkimusta ja magneettikuvantamista kasvaimen syvän invaasion arvioimisessa. Kahdellekymmenelle potilaalle tehtiin ennen leikkausta kolmiulotteinen ultraäänitutkimus sekä lantion magneettitutkimus, ja näiden tutkimusten tuloksia verrattiin sekä keskenään että leikkausnäytteiden histologisiin ominaisuuksiin. Magneettitutkimus todettiin näistä arvioiduista menetelmistä herkemmäksi (91,7 %) syvän invaasion osoittamisessa. Kolmiulotteinen ultraäänitutkimus oli kuitenkin

tarkempi (87,5 %). Näiden kuvantamismenetelmien peräkkäinen yhdistelmä oli osuvuudeltaan paras (80,0 %).

Kolmantena tavoitteena oli selvittää munasarjasyövän diagnostiikassa käytettyjen merkkiaineiden CA 12-5:n ja HE4:n soveltuvuutta endometriumkarsinooman levinneisyyden arviointiin. Näiden merkkiaineiden yhdistelmä oli soveltuvuudeltaan paras verrattuna sekä CA 12-5:n että HE4:n yksittäiseen käyttöön. Menetelmän herkkyys levinneen taudin ennustamisessa oli 57,1 %, tarkkuus 98,7 %, positiivinen ennustearvo 88,9 %, negatiivinen ennustearvo 92,6 % sekä osuvuus 92,2 %. Potilaan painoindeksi vaikutti HE4:n pitoisuuteen. Tämän sekoittavan tekijän mahdollisuus on otettava huomioon HE4-pitoisuuksia arvioitaessa.

Viimeisenä tämän tutkimuksen tavoitteena oli tutkia verisuonikasvutekijöiden merkitystä kasvaimen levinneisyyden ennustamisessa. Tutkimuksessa arvioitiin potilaiden seerumista mitatun verisuonten endoteliaalisen kasvutekijän VEGF:n, tämän liukoisen reseptorin sFLT-1:n sekä uuden verisuonikasvutekijän endogliinin pitoisuuksia verrattuna taudin levinneisyyteen. Lisäksi leikkausnäytteistä arvioitiin immunohistokemiallisilla värjäysmenetelmillä endogliinin, VEGF:n ja tämän solukalvon reseptoreiden VEGFR1:n ja VEGFR2:n ilmentymistä. Seerumin kohonneen VEGF-pitoisuuden todettiin liittyvän levinneeseen tautiin. Immunohistokemiallisella menetelmällä arvioidun kasvaimen verisuonitiheyden todettiin korreloivan kolmiulotteisella energiadopplerkuvantamisella määritetyn verisuonitiheyden kanssa.

Kasvaimen kohtuinvaasion arviointi endometriumin tilavuutta ja verenkiertoa mittaamalla voi helpottaa ennen leikkausta tehtävää levinneisyyden riskin arviointia. Tämän lisäksi CA 12-5:n ja HE4:n määritys voi olla hyödyllinen ja ei-invasiivinen tutkimus, joka auttaa leikkaushoidon suunnittelussa.

3. Table of contents

4. List of original communications

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I–IV).

I Saarelainen SK, Vuento MH, Kirkinen P, Mäenpää JU (2012): Preoperative assessment of endometrial carcinoma by three-dimensional power Doppler angiography. Ultrasound Obstet Gynecol;39:466–72.

II Saarelainen SK, Kööbi L, Järvenpää R, Laurila M, Mäenpää JU (2012): The preoperative assessment of deep myometrial invasion by three-dimensional ultrasound versus MRI in endometrial carcinoma. Acta Obstet Gynecol Scand;91:983–90.

III Saarelainen SK, Peltonen N, Lehtimäki T, Perheentupa A, Vuento MH, Mäenpää JU (2013): Predictive value of serum human epididymis protein 4 and cancer antigen 125 concentrations in endometrial carcinoma. Am J Obstet Gynecol;209:142.e1–6

IV Saarelainen SK, Staff S, Peltonen N, Lehtimäki T, Isola J, Kujala P, Vuento MH, Mäenpää JU. Endoglin, VEGF and its receptors in endometrial carcinoma. Submitted.

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5. Abbreviations

2D US	two-dimensional ultrasound
3D US	three-dimensional ultrasound
3D-PDA	three-dimensional power Doppler angiography
ACOG	the American College of Obstetricians and Gynecologists
ACR	the American College of Radiology
AEH	atypical endometrial hyperplasia
Akt	v-akt murine thymoma viral oncogene homolog 1
ALK1	activin A receptor type II-like1
ALK5	transforming growth factor β receptor 1
AUC	area under the curve
Bcl-2	B-cell lymphoma 2
BMI	body mass index (kg/m^2)
B-mode	brightness mode, two-dimensional ultrasound scan
CA125	cancer antigen 12-5, carbohydrate antigen 12-5
CA153	cancer antigen 15-3
CA199	cancer antigen 19-9, sialylated Lewis (a) antigen
CA724	cancer antigen 72-4
CD105	endoglin
CEA	carcinoembryonic antigen
CI	confidence interval
CT	computed tomography
CV	coefficient of variance
Da	dalton, unified atomic mass unit
EC	endometrial carcinoma
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbent assay
EORTC	European Organization for Research and Treatment of Cancer
ERBB2	erythroblastic leukemia viral oncogene homolog 2/HER2
ESMO	the European Society for Medical Oncology
FDG	2-[^{18}F]-fluoro-2-deoxy-D-glucose
FI	flow index
FIGO	International Federation of Gynecology and Obstetrics
FOS	FBJ murine osteosarcoma viral oncogene homolog

FOXO1	forkhead box protein O1
Gd-DOTA	gadolinium-tetraazacyclododecane tetraacetic acid
GPU	graphics processing unit
HE4	human epididymis protein 4
HIF-1α	hypoxia-inducible factor-1α
HNPCC	hereditary non-polyposis colorectal cancer
Hz	hertz ($1/s^{-1}$)
IGF-1	insulin-like growth factor 1
IU	international unit
KDR	kinase insert domain receptor, FLK-1
K-ras	Kirsten rat sarcoma viral oncogene homolog
M	molar concentration (mol/dm^3)
MAPK	mitogen-activated protein kinase
M-CSF	macrophage colony-stimulating factor
MRI	magnetic resonance imaging
MUC16	mucin 16 gene
MVD	microvessel density
NPV	negative predictive value
NSGO	Nordic Society of Gynecologic Oncology
OR	odds ratio
OVX1	ovarian cancer antigen X1
p53	tumor protein 53
PCOS	polycystic ovarian syndrome
PI3K	phosphatidylinositol-4,5 –bisphosphate 3-kinase
PIK3CA	phosphatidylinositol-4,5 –bisphosphate 3-kinase
PPV	positive predictive value
PRF	pulse repetition frequency
PTEN	phosphatase and tensin homolog
Pttg1	pituitary tumor-transforming gene 1
r	Spearman's rho
Ras	rat sarcoma oncogene
RMI	risk of malignancy index
ROC	receiver operating characteristics
ROI	region of interest
ROMA	risk of ovarian malignancy algorithm
SD	standard deviation
sFLT-1	soluble fms-like tyrosine kinase 1
Src	Rous sarcoma oncogene
Smad	mothers against decapentaplegic homolog
SUVmax	maximum standardized uptake value

T1	spin-lattice relaxation time
T2	spin-spin relaxation time
T	tesla (Wb/m^2)
TAF	tumor angiogenesis factor
TGF-β	transforming growth factor β
TDS	tumor-free distance to serosa
TE	echo time (ms)
TNF-α	tumor necrosis factor α
TR	repetition time (ms)
TSE	turbo spin echo
VCI	volume contrast imaging
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
VFI	vascularity flow index
VI	vascularity index
VIBE	volumetric interpolated breath hold examination
VOCAL	virtual organ computer-aided analysis
VOI	volume of interest
VPF	vascular permeability factor
WAP	whey-acidic protein
WFDC2	WAP four-disulfide core domain protein 2
WMF	wall motion filter

6. Introduction

Endometrial carcinoma is the most common gynecologic malignancy in developed countries. The treatment of endometrial carcinoma is surgical, with an adjuvant radio- or chemotherapy to a selected subgroup of patients. The surgical treatment includes a hysterectomy, bilateral salpingo-oophorectomy and peritoneal fluid sampling. To surgically stage the tumor, a pelvic and increasingly often also para-aortic lymphadenectomy is performed. Before 1988, the staging of endometrial carcinoma was clinical. Postoperative adjuvant therapies were recommended for patients with histopathological risk factors. This resulted in overtreatment exposing too many women to the deleterious effects of pelvic radiotherapy. Some were also undertreated, as occult para-aortic involvement was undetected. Due to the unreliability of the clinical staging system, International Federation of Gynecology and Obstetrics implemented a new surgical staging system in 1988 that involved a pelvic and para-aortic lymph node dissection (Creasman 1990). However, after 25 years, controversy still remains over the need for and extent of the lymph node dissection.

Several histopathological features of the tumor elevate the risk for lymph node metastases in endometrial carcinoma. These risk factors include deep (≥50%) myometrial invasion, large size of the tumor and extension of the tumor to the cervical stroma. In addition, poor histological differentiation raises the probability of lymph node metastases. In theory, all of these features are evaluable preoperatively. Tumor invasion, size and extension can be assessed by imaging methods and histologic differentiation can be evaluated from the endometrial biopsy or curettage specimen upon which the initial diagnosis of a malignancy is based. However, the accuracy of current imaging methods, i.e. sonography, magnetic resonance imaging and computed tomography, in detecting deep invasion or cervical spread is not consistent.

The objective for lymphadenectomy is not therapeutic. Instead, complete surgical staging by removal of the inner genitalia and pelvic with/without para-aortic lymph nodes directs the postoperative adjuvant therapy for patients that have a high risk for disease recurrence and thus a poorer prognosis. However, performing a lymphadenectomy carries a potential risk of morbidity intra- and postoperatively. The dissection causes an increase in operation time, thus exposing the patients to a greater risk for infections. Blood loss may also increase. Probably

the most distressing side effect is the evolvement of lower limb lymphedema and intra-abdominal lymphocysts. These incidents may have a major detrimental effect on patients' quality of life.

The intention of the present study was to investigate new modalities in predicting preoperatively the presence of metastases in endometrial carcinoma. With a more accurate preoperative risk assessment, unnecessary lymphadenectomies with potential side effects could be avoided. The first objective was to investigate three-dimensional sonography with and without magnetic resonance imaging in the preoperative assessment of deep myometrial invasion. In addition, the predictive value of tumor markers HE4 and CA 125 was evaluated with respect to the presence of metastases. Finally, the metastastic potential of endometrial carcinoma was evaluated by examining angiogenic markers from preoperative serum samples and hysterectomy specimens.

7. Review of the literature

7.1 Endometrial carcinoma

Endometrial carcinoma (EC), or carcinoma of the corpus uteri, is the sixth most common cancer in women worldwide, with an estimated 287 000 new cases per year. In developed countries, with 142 000 new cases annually, endometrial carcinoma is the most common gynecologic malignancy, causing 33 000 deaths every year (Ferlay et al. 2010, Jemal et al. 2011). As endometrial carcinoma is partly associated with general socioeconomic status, the global incidence is expected to rise as the population in developing countries adopts a more westernized lifestyle along with its detrimental health effect, primarily due to dietary factors (World Health Organization 2008). The lifetime cumulative risk for a woman to develop an endometrial carcinoma is 1.0% worldwide (0–74 years) (Jemal et al. 2011). The median age of patients at the time of diagnosis is 61 years, and ninety percent of them are older than 50 years (Creasman et al. 2001).

Endometrial carcinoma by definition is a malignant tumor of the endometrium. It is divided into two types according to the histologic characteristics. Type I endometrial carcinoma is an endometrioid adenocarcinoma that is related to endo- or exogenous estrogen exposure. Type I endometrial carcinoma comprises three subtypes that are categorized by their histology into well (grade 1), moderately (grade 2) and poorly (grade 3) differentiated tumors. As obesity becomes more common, the incidence of type I endometrial carcinoma is expected to rise, because of the production of estrone by adipose tissue. Typically, type I endometrial carcinoma develops in perimenopausal, obese women with an endometrial hyperplasia as a precursor (Sorosky 2008, World Health Organization 2008).

Type II endometrial carcinoma is not related to estrogen exposure, occurs typically in older women and generally has a much poorer prognosis than type I disease. Type II carcinoma includes papillary serous or clear cell subtypes and may exhibit a mixed endometrioid component (Huang et al. 2007, Slomovitz et al. 2003). Endometrial carcinosarcoma, which is a poorly differentiated metaplastic carcinoma by its histological properties, is included in type II endometrial carcinoma, as the course of the disease and treatment are similar to poorly

differentiated endometrioid carcinoma and type II cancers (Amant et al. 2005, Bokhman 1983, McCluggage 2002, Sorosky 2008).

Exhibiting hereditary features, a familial type of endometrial carcinoma occurs ordinarily in patients with a hereditary non-polyposis colorectal cancer (HNPCC) referred to as Lynch II syndrome. The hereditary types constitute 10% of all endometrial carcinomas, and they are occasionally referred to as type III cancers. Although Lynch II syndrome is a major predisposing factor for the hereditary or genetic endometrial carcinomas, it is purported to be involved in only 50% of them (Sorosky 2008).

The prognosis of endometrial carcinoma is generally good, as 75% of the tumors are diagnosed before metastases occur (Sorosky 2008). Prognostically, the lymph node status is the most important factor influencing survival (Creasman et al. 2006, Mariani et al. 2001). Other prognostic factors, increasing the risk for nodal metastases, include size of the tumor, histologic differentiation (grade), myometrial invasion and cervical spread of the tumor (Abeler and Kjorstad 1991, Creasman et al. 1987, Mariani et al. 2002, Schink et al. 1991, Shah et al. 2005). Lymphovascular space involvement regardless of the lymph node status, and DNA aneuploidy are also found to be associated with a worsened prognosis (Ambros and Kurman 1992, Baak et al. 1995, Gal et al. 1991, Kodama et al. 1996). In a carcinoma confined to the uterus, the five-year survival is 85.5–91.1%. If lymph node metastases are present at the time of the diagnosis, the survival decreases to 57.3%. Patients with distant metastases carry the gravest prognosis, with a five-year survival of 20.1% (Creasman et al. 2006).

Endometrial carcinoma is staged surgically. Because the classical predictors for metastases, histological grade and the degree of myometrial invasion, were not accurate for treatment planning, in 1988 International Federation of Gynecology and Obstetrics (FIGO) adopted a new system that changed the staging from clinical to surgical. Surgical staging includes the removal of the inner genitalia, pelvic with (or without) para-aortic lymphadenectomy and peritoneal fluid sampling. The staging system was renewed in 2009 to better represent clinical practice (Creasman 1990, Mutch 2009) (Table 1).

Table 1. FIGO 1988 and 2009 staging of endometrial carcinoma

Description	Stage	
	1988	2009
Tumor confined to the uterus	I	I
No myometrial invasion	IA	IA
Myometrial invasion less than 50%	IB	IA
Myometrial invasion 50% or greater	IC	IB
Cervical involvement	II	II
Endocervical glandular involvement only*	IIA	-
Tumor extends to the cervical stroma	IIB	II
Tumor extends to the uterine serosa, adnexae, lymph nodes or vagina	III	III
Serosal or adnexal involvement or positive peritoneal cytology†	IIIA	IIIA
Vaginal and/or parametrial involvement‡	IIIB	IIIB
Metastases in pelvic lymph nodes	IIIC	IIIC1
Metastases in para-aortic lymph nodes	IIIC	IIIC2
Tumor extends to the abdominal organs and/or has distant metastases	IV	IV
Tumor extends to the bladder or rectum	IVA	IVA
Metastases in abdominal organ parenchyma or extra-abdominal metastases, including inguinal lymph nodes	IVB	IVB

*According to the FIGO 2009 staging, an isolated endocervical glandular involvement is considered as Stage I. †In the FIGO 2009 staging, a positive peritoneal cytology is reported separately without changing the stage. ‡In the FIGO 1988 staging there was no stage for parametrial involvement.

7.1.1 Risk factors

Endometrial carcinoma most frequently occurs in postmenopausal women. Characteristic of malignant tumors, it is a multifactorial disease. The most important predisposing factor for the development of an endometrial malignancy is exposure to estrogen unopposed by progestins. Adipose tissue is the main source of estrogen in postmenopausal women, and the circulating level of estrogen is elevated in obese postmenopausal women compared to their normal weight counterparts (Cauley et al. 1989). Closely concomitant to obesity, insulin resistance in diabetes mellitus type 2 has been shown to be independently associated with an elevated risk for endometrial carcinoma (Mu et al. 2012). The interaction between insulin, insulin receptors, and insulin-like growth factor 1 (IGF-1) is purported to participate in the carcinogenic process. In addition, insulin directly stimulates cell proliferation and inhibition of apoptosis by activating the PI3K/Akt and

Ras/MAPK pathways (Chang et al. 2013, Lathi et al. 2005, Nagamani and Stuart 1998, Nout et al. 2012).

Medical conditions producing a continuous unopposed estrogen exposure contribute to the risk for developing endometrial carcinoma. These conditions include polycystic ovarian syndrome (PCOS), infertility related to anovulation, liver cirrhosis and estrogen producing tumors (Sorosky 2008). Exogenous estrogen in a hormone replacement therapy, not accompanied by progestin, promotes the risk of endometrial carcinoma sixfold (Weiderpass et al. 1999). Tamoxifen, used mainly for the chemoprevention and postoperative adjuvant treatment of breast cancer, induces estrogen-regulated genes and promotes endometrial cell growth. Tamoxifen intake predisposes endometrial changes that are commonly benign, but malignant transformation to endometrial carcinoma has been also shown to occur (Fisher et al. 1994, Fisher et al. 1998, Marchesoni et al. 2001). The carcinogenic effect of tamoxifen on the endometrium is probably only partly mediated via estrogen-regulated genes, as it has been also shown to induce oncogenic mutations in the K-ras gene (Wallen et al. 2005).

Patients with HNPCC constitute the main proportion of women with a hereditary susceptibility for endometrial carcinoma. In addition, mutations of the genes encoding PTEN, FOXO1, PIK3CA, E-cadherin, β-Catenin, K-ras and p53 have been associated with endometrial pathology (Ellis and Ghaem-Maghami 2010, Garcia-Dios et al. 2013). The carriers of germline mutations in BRCA1 and BRCA2 genes, predisposing factors for breast, ovarian, Fallopian tube and peritoneal cancers, do not have a greater risk for developing an endometrial cancer (Beiner et al. 2007, Levine et al. 2001). As an exception to this, the incidence of papillary serous carcinoma of the endometrium may be increased among BRCA carriers (Lavie et al. 2000, Lavie et al. 2004). The risk for developing an endometrial carcinoma is twofold in white women when compared to African-American women. However, African-American women appear to have a poorer prognosis. The cause for this is unclear, but possible explanations are genetic susceptibility to the more aggressive subtypes of cancer, limited access to health care, and insurance policies (Hill et al. 1995).

7.1.2 Diagnosis

Postmenopausal or irregular bleeding is the most common symptom that leads to a diagnosis of an endometrial carcinoma. If postmenopausal bleeding occurs, it usually triggers investigations to rule out a malignancy. The diagnostic pitfall lies with those premenopausal women that have irregular bleeding, a symptom often interpreted to be a sign of forthcoming menopause. The preoperative diagnosis is

based on the histopathological evaluation of an endometrial biopsy or curettage specimen. To rule out endometrial pathology, atypical glandular cells in a cytologic screening in women over age 35 should be verified by an endometrial biopsy (Schnatz et al. 2006, Sharpless et al. 2005). Atypical endometrial hyperplasia (AEH) is a known precursor of carcinoma and up to 59% of women with an AEH may have a coexisting cancer. Therefore a dilatation and curettage is warranted if an endometrial biopsy is evaluated to represent AEH (Antonsen et al. 2012, Chen et al. 2013). Although several studies have evaluated the accuracy of various non-invasive, primarily sonographic, imaging methods for the diagnosis of endometrial cancer, the results do not advocate the alteration of the present practice, i.e. the histopathological diagnosis remains as the gold standard (Alcázar and Galván 2009, Clark et al. 2002, Develioglu et al. 2003, Epstein et al. 2002, Mercé et al. 2007, Odeh et al. 2007, Opolskiene et al. 2009, Opolskiene et al. 2010, Smith-Bindman et al. 1998, Tabor et al. 2002, Van den Bosch et al. 1995).

7.1.3 Treatment

Surgical treatment

The cornerstone of the treatment of endometrial carcinoma is surgery, including hysterectomy, bilateral salpingo-oophorectomy, and peritoneal fluid sampling. In the case of a serous papillary carcinoma, an infracolic omentectomy and peritoneal biopsies are also recommended. The accurate staging of endometrial carcinoma warrants lymph node dissection from the pelvic and para-aortic regions, but the indications and extent of the dissection is an issue around which controversy remains. Prospective studies have shown no survival benefit of lymphadenectomy in early-stage endometrial carcinoma (ASTEC study group 2009, Benedetti Panici et al. 2008). The therapeutic effect of lymph node dissection has been demonstrated in retrospective studies that have potential sources of bias and, except in the study by Kilgore et al. (1995), in patients with an advanced-stage disease or moderate to high risk for recurrence (Chan et al. 2006, Cragun et al. 2005, Lutman et al. 2006, Todo et al. 2010a). In theory, the impact on survival is mediated by preventing the recurrence of the disease as cancerous lymph nodes are dissected. Despite the unestablished therapeutic role of lymphadenectomy, it is at present the only reliable method of evaluating the lymph node status, providing important prognostic information and assisting in the tailoring of adjuvant therapy for the individual patient. However, the removal of the lymphatic tissue from the pelvic and para-aortic regions is not devoid of adverse effects. Longer operation

time may cause increased blood loss and risk for postoperative infections. Late-onset complications may occur, most commonly lower extremity lymphedema and the development of intra-abdominal lymphocysts. Lymphocysts carry an increased risk for fostering infections, whereas lymphedema at worst can have a serious detrimental influence on the patients' quality of life. According to the present literature, the incidence of postoperative lower extremity lymphedema and lymphocyst formation ranges from 1–38% and 0–19%, respectively (Abu-Rustum et al. 2006, Backes et al. 2012, Barnett et al. 2011, Bell et al. 2008, Ghezzi et al. 2012, Hahn et al. 2010, Konno et al. 2011, Nunns et al. 2000, Todo et al. 2010b). Open surgery with pelvic and para-aortic lymphadenectomy carries the greatest risk for lymphatic system complications (Konno et al. 2011, Todo et al. 2010a).

Surgical therapy may be via laparotomy or laparoscopy. Laparoscopic-assisted vaginal hysterectomy or total laparoscopic hysterectomy have been recommended as less invasive and more tolerated by patients. In the studies comparing open surgery and laparoscopy, the minimally invasive approach has proven to be safe and cost-effective (Fram 2002, Malzoni et al. 2009, Mourits et al. 2010, Walker et al. 2012, Zorlu et al. 2005, Zullo et al. 2009). Pelvic lymphadenectomy can be performed laparoscopically, whereas para-aortic lymph node dissection is challenging via standard laparoscopy and is performed in selected institutions only. Recently, robotic-assisted laparoscopy has been introduced in the treatment of endometrial carcinoma. With robotic-assisted laparoscopy, the lymph node dissection can be extended to the para-aortic area without exposing the patient to the hazards of invasive open surgery (Brudie et al. 2013, Cardenas-Goicoechea et al. 2010, Kilgore et al. 2013). However, the costs of the robotic system limit its universal application in the treatment of endometrial carcinoma.

Adjuvant therapies

The adjuvant therapy of endometrial carcinoma consists of radiation or chemotherapy that can be administered as alternatives to each other or in combination. According to the result of the postoperative histopathological report, the patient's risk for disease recurrence is evaluated. Usually, this is accomplished by classifying the tumor into one of the following categories: low-risk, intermediate-risk or high-risk for recurrence (Table 2). Patients with a low-risk tumor have a good prognosis and do not benefit from adjuvant therapy (Elliott et al. 1994, Sorbe et al. 2009). The adjuvant treatment of intermediate-risk and high-risk patients differ according to local practices. Two large prospective studies, the Gynecologic Oncology Group (GOG) study, and the PORTEC study, failed to demonstrate survival benefit from postoperative pelvic radiation in patients with an

intermediate-risk endometrial carcinoma, despite the rate for local recurrences being lower in the treatment arm. Based on the slight, albeit not statistically significant, survival benefit, both study groups resulted in the recommendation of postoperative radiotherapy to patients with a high-intermediate risk disease (based on patient's age and histopathological features) (Creutzberg et al. 2000, Keys et al. 2004). The results of studies comparing chemotherapy and radiotherapy in the postoperative treatment of high-risk or advanced stage disease are not in agreement. In a Japanese trial comparing external beam radiotherapy and chemotherapy, no survival benefit over the other was seen in either of the treatment arms (Susumu et al. 2008). A similar outcome was found in a trial by Maggi and colleagues (2006).

Table 2. Postoperative risk categories

Risk category	Histology	Other
Low	Stage IA G 1–2	Patient's age, the size of the tumor and the presence of lymphovascular invasion may upgrade the risk category. These criteria are implemented according to institutional guidelines
Intermediate	Stage IA G 3 Stage IB G 1–2	
High	Stage IB G 3 Stage II–IV Non-endometrioid histology	

A GOG trial by Randall and associates (2006) also compared chemotherapy and whole-abdomen radiotherapy in the treatment of stage III and IV endometrial carcinoma. They found that the treatment with chemotherapy resulted in a better five-year survival (55% for chemotherapy and 42% for radiotherapy), with the cost of significantly increased toxicity. The combination of chemotherapy and radiotherapy appears to be a acceptable choice for patients with a high risk for recurrence. However, studies evaluating the efficacy of the combined treatment versus radiotherapy alone have resulted in conflicting outcomes. The studies by Kuoppala et al. (2008), Morrow et al. (1990) and Secord et al. (2013) did not show benefit from the combination. In contrast to these studies, in the Nordic Society of Gynecologic Oncology/European Organization for the Research and Treatment of Cancer (NSGO/EORTC) study, the combination of the treatments was associated with a 36% reduction of disease relapse or death (Hogberg et al. 2010). A similar finding was made by Lee and Viswanathan (2012) who found that combining chemotherapy with radiotherapy improved the five-year overall survival rate in

women with node-positive endometrial cancer when compared to radiotherapy alone (90% versus 67%, respectively).

Postoperative vaginal brachytherapy is a treatment option for intermediate-risk patients. The PORTEC-2 study evaluated the feasibility of brachytherapy compared to external pelvic radiotherapy. Vaginal brachytherapy was found to have less gastrointestinal toxicity than external radiotherapy, whereas the efficacy of the prevention of the local recurrences was similar in both treatment arms (Nout et al. 2010).

The role of hormonal therapy with progestagens in endometrial carcinoma is established in palliative treatment. However, randomized controlled trials have failed to demonstrate a survival benefit of hormonal therapy as a postoperative adjuvant regimen (De Palo et al. 1993, Macdonald et al. 1988, Vergote et al. 1989).

7.2 Preoperative assessment of endometrial carcinoma

The aim of the preoperative assessment of endometrial carcinoma is to acquire information that assists in tailoring optimal treatment for an individual patient. Whereas surgery is the standard treatment of endometrial cancer, the results of the preoperative imaging, blood samples, or histopathological evaluation may help deciding the extent of the operation.

Preoperative imaging may have two different purposes. It can be used in evaluating myometrial invasion or in extrauterine spread. The latter cannot be accomplished by transvaginal sonography, as evaluation of the lymph node status and abdominal organs is impossible. Abdominal sonography is more feasible in this setting, but is highly operator-dependent and still lacks sufficient accuracy. Thus, methods that can scan the whole abdomen and preferably the entire body area are, in theory, the only reasonable options for preoperative staging. However, transvaginal sonography is not non-significant. Histopathological studies have indicated that deep (≥50%) myometrial invasion or cervical involvement of the tumor magnify the risk for advanced stage disease three- to fourfold (Creasman et al. 1987, Creasman et al. 1999, Geels et al. 2013). If accurate enough, evaluation of the myometrial invasion and cervical spread by sonography may assist in the preoperative risk assessment. However, it should be noted that the currently existing guidelines for the diagnosis, treatment and follow-up of endometrial carcinoma are not in consensus regarding preoperative imaging. The Asian Oncology Summit statement and the American College of Obstetricians and Gynecologists (ACOG) Practice Bulletin do not support the use of preoperative imaging as a staging procedure or in the evaluation of myometrial invasion (American College of Obstetricians and Gynecologists 2005, Tangjitgamol et al.

2009). The European Society for Medical Oncology (ESMO), the Japan Society of Gynecologic Oncology (JSGO) and the American College of Radiology (ACR) state that imaging, preferably magnetic resonance imaging (MRI), can be used in the preoperative workup of patients (Colombo et al. 2011, Lee et al. 2011, Nagase et al. 2010).

Besides providing the diagnosis of an endometrial carcinoma, the result of the histopathologic evaluation of endometrial biopsy or curettage specimen influences the operative treatment. Poorly differentiated or non-endometrioid histology warrants extensive surgery with a lymphadenectomy. In addition to imaging and histology, serum markers can be used in the planning process.

7.2.1 Two-dimensional sonography

The first study to examine the feasibility of sonography in the evaluation of myometrial invasion in endometrial carcinoma was by Obata and associates (1985). They used an intrauterine radial scanner to evaluate endometrial pathology. Nevertheless, the intrauterine technique was invasive and not adopted in clinical practice. The first study utilizing a transabdominal approach was by Fleischer and colleagues (1987). In their study of 20 patients, they reported a sensitivity of 100% in detecting deep myometrial invasion, whereas specificity was 75%. They stated that the assessment of myometrial invasion by sonography was feasible. Transabdominal sonography was assessed in this setting by other following studies that confirmed the results of Fleischer et al. (Cacciatore et al. 1989b, Gordon et al. 1989, Lehtovirta et al. 1987). Transvaginal sonography was also available and promptly adopted in the imaging of endometrial carcinoma (Cacciatore et al. 1989a, Conte et al. 1990, Cruickshank et al. 1989, Gordon et al. 1990). The transvaginal approach enables the near proximity of the probe to the investigated organ and the use of high frequencies, resulting in an improved ultrasound image. However, transvaginal sonography may not be the primary examination method in some parts of the world. Instead, a transabdominal approach is chosen.

In the first studies of the evaluation of myometrial invasion the degree of invasion was graded in various manners. Some investigators preferred the use of percentiles or a 50/50 division (Fleischer et al. 1987, Gordon et al. 1989), and others categorized the invasion into 1/3, 2/3 and 3/3 of the myometrium (Cacciatore et al. 1989a, Cacciatore et al. 1989b, Gordon et al. 1990, Lehtovirta et al. 1987, Obata et al. 1985). The first study to use a classification corresponding to the new FIGO 1989 classification of myometrial invasion (less than 50%-more than 50%) was by Karlsson and colleagues (1992). They found that a transvaginal ultrasound examination had a sensitivity of 78.9% and specificity of 100% in

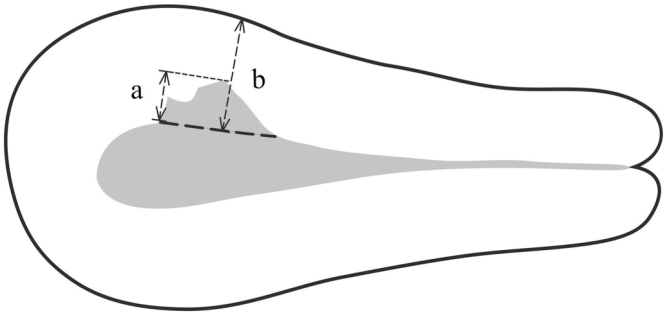
detecting deep myometrial invasion. The positive predictive value and negative predictive value were 100% and 73.3%, respectively. When appraising the studies regarding the assessment of myometrial invasion, one has to decide which summary statistic is considered the most relevant. If the primary supposition is that extensive surgery, with a lymphadenectomy, should be performed at all times except when preoperative imaging of the myometrium shows no signs of deep invasion, the importance of the negative predictive value is accentuated. Alternatively, the sensitivity is the principal statistics when the lymph node dissection is performed only on patients with an indication of deep invasion in the preoperative imaging (Altman and Bland 1994). Naturally, these assumptions only exist if the results of the histopathological evaluation or other preoperative assessment do not favor a lymphadenectomy.

The assessment of cervical invasion of the tumor is performed simultaneous to the evaluation of myometrial invasion. It should be noted that the change in the FIGO staging system that took place in 2009 greatly influenced to the assessment of the cervix in endometrial carcinoma. The former stage IIA, with a glandular or superficial infiltration of the tumor, was excluded from the new staging system. The old stage IIB, with a stromal invasion, was regarded as the new stage II (Creasman 1990, Mutch 2009) (Table 1).

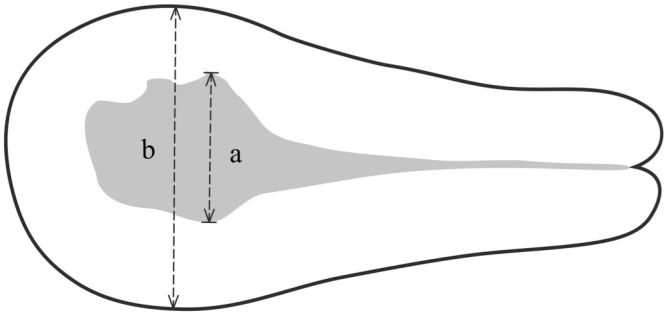
Since the early trials, the sonographic assessment of endometrial carcinoma has been widely investigated. The greatest distinction between the pioneering and contemporary studies is the technical evolution of the ultrasound machines. Ultrasound units are equipped with multifrequency probes that provide improved resolution and image-enhancing algorithms that require up-to-date computer technology. However, the technique of the assessment has not been changed. There are at least three different methods for evaluating the depth of myometrial invasion, and all have advantages and disadvantages. In the first method, first published by Fleischer et al. (1987), the margin of the normal endomyometrial junction has to be estimated to create a reference line for the evaluation of the invasion (Figure 1A). This may be a major source of bias. On the other hand, an invasion of an asymmetrical tumor (distributed unevenly along the walls of the uterine cavity) is evaluable. The second technique, introduced by Karlsson et al. (1992), is not so operator-dependent, but it assumes that the endometrial tumor is relatively symmetrically distributed inside the uterine cavity (Figure 1B). The third method presents an alternative point of view, as the degree of invasion is evaluated by measuring the myometrial tumor-free distance to the serous margin of the uterus (Figure 1C). Recent histopathological studies suggest that tumor-free distance could be a better predictor for an advanced stage disease than evaluating the myometrial invasion conventionally (Chattopadhyay et al. 2012, Kondalsamy-Chennakesavan et al. 2010, Lindauer et al. 2003, Schwab et al. 2009).

Figure 1. Schematic illustrations of sonographic sagittal planes of the uterus with an endometrial tumor. A. The depth of invasion is calculated by dividing the approximated tumor extension by the thickness of the myometrial wall (a/b). B. The depth of invasion is calculated by dividing the maximal thickness of the tumor by the antero-posterior diameter of the uterus. C. The shortest tumor-free distance to the serosa is measured.

A.



B.



C.

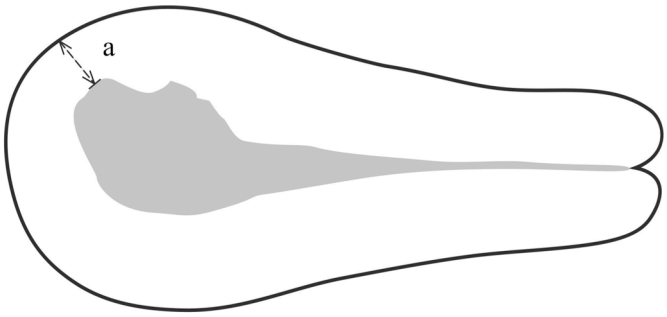


Figure by the author

However, only a single published sonographic study by Alcázar and colleagues (2009) utilizes this method, in which the measurements are performed by three-dimensional sonography only.

The performance of transvaginal sonography in the detection of deep myometrial invasion or cervical involvement varies greatly in the published literature. The sensitivity for detection has ranged from 5 to 100% [mean (SD), 77.2% (18.7)], whereas the specificity of the method has ranged from 50 to 100% [mean (SD), 80.9% (11.3)]. The positive predictive value, negative predictive value and overall accuracy of sonography have been 50–100%, 65–100% and 60–99%, respectively [mean (SD), 74.6% (15.1); 85.1% (9.5); 78.9% (7.9), respectively] (Table 3). The considerable variation in the performance of the method is partly a result of the differing measuring principles used and partly probably due to technical differences in the study settings. The majority of the studies relied on the examiner's subjective measurement of the invasion (Figure 1A) (Akbayir et al. 2011, Akbayir et al. 2012, Arko and Takac 2000, Artner et al. 1994, Berretta et al. 2008, Cacciatore et al. 1989a, Cacciatore et al. 1989b, Cagnazzo et al. 1992, Cheng et al. 1998, DelMaschio et al. 1993, Fleischer et al. 1987, Gabrielli et al. 1996, Gordon et al. 1989, Gordon et al. 1990, Kanat-Pektas et al. 2008, Kim et al. 1995, Köse et al. 2003, Mascilini et al. 2013, Lehtovirta et al. 1987, Obata et al. 1985, Olaya et al. 1998, Savelli et al. 2008, Savelli et al. 2012, Shipley et al. 1992, Takac 2007, Yamashita et al. 1993b, Ørtoft et al. 2013). Others were more objective, using a quantitative method (Figure 1B) (Alcázar et al. 1999, De Smet et al. 2006, Karlsson et al. 1992, Prömpeler et al. 1994, Weber et al. 1995). In the studies by Antonsen et al. (2013b), Sykes et al. (2002), van Doorn et al. (2002) and Özdemir et al. (2009), the method used in the measurement of invasion was not clearly defined.

Some previous studies also assessed the cervical involvement of the tumor. In fact, only three studies have focused on the detection of cervical involvement only (Çelik et al. 2010, Cicinelli et al. 2008, Kietlinska et al. 1998). The sonographic characteristics of a cervical involvement include irregularity of the endomyometrial junction and possibly distention of the endocervical canal. During the examination, the operator may apply a slight pressure on the probe to exclude a protrusion of the intracavitary tumor to the cervical canal from an actual infiltration of the cancer (Savelli et al. 2008). When the results of the studies are evaluated, the sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of transvaginal sonography in the detection of cervical involvement are 19–93%, 63–100%, 25–100%, 81–98% and 65–98%, respectively [mean (SD), 64.4% (20.4); 89.1% (10.7); 61.3% (26.5); 92.8% (5.3); 85.3% (10.1), respectively] (Table 5). As with the studies of detection of myometrial invasion, the results fluctuate remarkably suggesting that the assessment of cervical spread is challenging.

Table 3. Performance of sonographic methods in detecting deep (≥50%) myometrial invasion in endometrial carcinoma.

Author	Year	Patients	Method	Sensitivity	Specificity	PPV	NPV	Accuracy
2D								
Akbayir	2011	298	TV	68	82	65	84	78
Akbayir	2012	219	TV	62	81	60	82	75
Alcázar	1999	50	TV	87	94	87	94	92
Antonsen	2013	194	TV	71	72	51	86	72
Arko	2000	120	TV	79	69	63	83	73
Artner	1994	69	TV	100	97	97	100	99
Berretta	2008	75	TV	62	79	54	82	73
Cacciatore	1989a	93	TA	N/A	N/A	N/A	N/A	80
Cacciatore	1989b	23	TV	N/A	N/A	N/A	N/A	87
Cagnazzo	1992	14	TV	80	77	87	66	78
Cheng	1998	42	TV	88	100	100	93	81
Conte	1990	20	TV	N/A	N/A	N/A	N/A	90
De Smet	2006	97	TV	61	86	N/A	N/A	N/A
DelMaschio	1993	42	TV	86	65	73	81	76
Fleischer	1987	20	TA	100	75	50	100	80
Gabrielli	1996	67	TV	88	71	66	91	78
Gordon	1989	15	TA	100	50	N/A	N/A	79
Kanat-Pektas	2008	120	TV	62	75	66	71	69
Karlsson	1992	30	TV	79	100	100	73	87
Kim	1995	26	TV	50	81	63	72	69
Köse	2003	47	TV	92	82	95	93	85
Lehtovirta	1987	24	TA	N/A	N/A	N/A	N/A	79
Mascilini	2013	144	TV	77	81	N/A	N/A	N/A
Obata	1985	32	IU	N/A	N/A	N/A	N/A	82
Olaya	1998	50	TV	94	85	76	97	88
Prömpeler	1994	96	TV	93	71	73	93	81
Savelli	2008	74	TV	84	83	79	88	84

Savelli	2012	155	TV	75	89	86	79	81
Shipley	1992	50	TV	58	92	70	88	84
Sykes	2002	108	TV	5	100	100	65	66
Takac	2007	53	TV	86	76	80	83	81
van Doorn	2002	93	TV	79	72	61	86	74
Weber	1995	80	TV	89	83	73	94	60
Yamashita	1993b	40	TV	77	93	83	89	68
Ørtoft	2012	156	TV	77	72	69	79	74
Özdemir	2009	64	TV	85	75	61	92	78
3D								
Alcázar	2009	107	TDS	100	61	50	100	72
			SA	93	83	68	97	85
Mascilini	2013	144	TUr	60	85	73	75	74
			TUV	68	67	59	75	67
Jantarasaengaram	2013	60	VCI	100	90	79	100	93

2D, two-dimensional sonography. 3D, three-dimensional sonography. IU, intrauterine. NPV, negative predictive value. PPV, positive predictive value. SA, subjective assessment. TA, transabdominal. TDS, tumor-free distance to serosa. TUr, tumor/uterine volume ratio. TUV, tumor volume. TV, transvaginal. VCI, volume contrast imaging.

A recent statement of the International Endometrial Tumor Analysis (IETA) group addresses the definition of the sonographic characteristics of endometrial lesions. The objective of the statement is to standardize the terminology in a manner that is applicable to clinical practice. However, although the statement presents valuable information on the sonographic features of the endometrium and the conduct of a gynecologic ultrasound examination, it does not take a stance on the assessment of myometrial invasion or cervical spread in the case of endometrial carcinoma (Leone et al. 2010).

As in ultrasound examinations generally, factors that influence the image quality can hamper the accuracy of the evaluation. In gynecologic sonography, the major factors that complicate the scan are the obesity of the patient and fibroids of the uterus. Large, polypotic tumors that stretch the myometrial wall may cause overestimation of the invasion. In addition, calcificated arcuate vessels of the myometrium may cause signal attenuation and impairment of the image. Regarding endometrial carcinoma, most of the patients are postmenopausal and sometimes have marked comorbidities. The insertion of the transvaginal probe may be unachievable due to vaginal strictures or pain. Furthermore, the lithotomy position that is usually used in a transvaginal sonography may prove to be difficult for some elderly patients, although the required time for the examination is not long.

7.2.2 Magnetic resonance imaging

MRI is well-suited for the assessment of malignant tumors, as it provides safe, non-radiating imaging modality with a good soft tissue contrast and the ability to view multiplanar images. In endometrial carcinoma, MRI is the most common imaging modality used for preoperative assessment worldwide. In contrast to transvaginal sonography, MRI enables the evaluation of the extrapelvic extension of the endometrial tumor, as the assessment of lymph nodes and intraperitoneal organs is achievable.

The first publication of the assessment of endometrial carcinoma by MRI was by Bryan and colleagues (1983). In this series of cases of pelvic conditions, the authors presented three cases with an endometrial carcinoma. The authors stated that compared to computed tomography (CT), MRI lacked sufficient tissue discrimination (Bryan et al. 1983). One of the first large studies on preoperative assessment was by Hricak and colleagues (1987). They evaluated the preoperative MRI scans of 51 women with a suspected endometrial pathology. The accuracy of the method in the preoperative staging and evaluation of the myometrial invasion was 92% and 82%, respectively. In the 1980s, MRI was still at its inception and since, as in sonography, the technical progress has been extensive.

In the published literature, both the sensitivity and specificity of MRI in detecting deep myometrial invasion have ranged from 50 to 100% [mean (SD), 80.4% (13.0) and 84.0% (12.5), respectively]. The reported positive predictive values and negative predictive values have varied between 42–100% and 49–100%, respectively [mean (SD), 76.8% (16.5) and 86.5% (9.9), respectively]. The overall accuracy of MRI has ranged from 58 to 98% [mean (SD), 81.9% (10.1)] (Table 4). The evaluation of cervical spread of the tumor is found to be relatively feasible by MRI, with a sensitivity, specificity, positive predictive value and negative predictive value of 19–90%, 86–100%, 46–100%, 85–98%, respectively [mean (SD), 62.0 (24.8); 92.8 (5.4); 68.4 (18.9) and 92.0 (4.4), respectively]. The reported accuracy of the method in detecting cervical spread has varied between 46% and 98% [mean (SD), 83.2 (15.1)] (Table 5).

MRI protocols for the assessment of myometrial invasion and cervical spread vary according to the institution. At least one T1-weighted and two T2-weighted image sequences are most commonly used. The disruption of the junctional zone in T2-weighted image sequences is described to be a typical landmark for a myometrial invasion (Yamashita et al. 1993a). However, this sign may not be visible in premenopausal women, especially in the first days of the menstrual cycle. Contrast agents, most commonly based on gadolinium (Gd), enhance the accuracy of MRI imaging. After intravenous administration of a contrast agent, the imaging protocols range from a single image sequence up to eight dynamic imaging acquisitions. Contrast-enhanced MRI sequences are recommended when evaluating atrophic uteri, patients with a suspected adenomyosis or fibroids, or when an advanced stage is suspected with an invasion to the bladder or rectal walls (Kinkel et al. 2009). The assessment of the endomyometrial junctional zone is enhanced by T1-weighted contrast-enhanced images, as the normal endometrium enhances more than a tumoral endometrium (Saez et al. 2000, Sironi et al. 1992a).

In a similar manner as in the sonographic evaluation of endometrial carcinoma, the pitfalls in the magnetic resonance imaging are those pathologic conditions that affect or stretch the myometrial wall, most commonly polypoid tumors, adenomyosis, and large fibroids. In addition, MRI acquisition is time-consuming (sometimes up to 40 minutes) causing susceptibility to artifacts caused by abdominal wall movements or peristalsis (Sanjuan et al. 2008). Other sources of bias are magnetic susceptibility, chemical shift and dielectric effect (Torricelli et al. 2008). The supine position inside the scanner can be unpleasant especially for people that are claustrophobic. The larger bores of modern scanners may diminish the risk for claustrophobic attacks.

Table 4. Performance of cross-sectional imaging methods in detecting deep (≥50%) myometrial invasion in endometrial carcinoma.

Author	Year	Patients	Method	Sensitivity	Specificity	PPV	NPV	Accuracy
MRI								
Antonsen	2013	227	1.5T	87	57	44	92	66
Cagnazzo	1992	30	0.5T	87	78	82	84	83
Cunha	2001	40	1.0T	80	100	100	89	93
DelMaschio	1993	42	0.5T	91	75	80	88	83
Gordon	1989	15	0.5T	100	50	N/A	N/A	71
Hori*	2009	30	3.0T	70/70	85/95	70/88	85/86	80/87
			1.5T	80/60	85/85	73/67	89/81	83/77
Hricak	1987	39	0.5T	100	97	83	100	97
Hwang	2009	53	1.5T	50	90	64	83	79
Kang	2012	122	1.5T	N/A	100	N/A	95	95
Kim	1995	26	0.5T	90	88	82	93	89
Lien	1991	33	1.5T	91	64	83	78	82
Ortashi	2008	100	1.0/1.5T	56	86	43	91	81
Rockall†	2007	96	1.5T/T1	72	88	72	88	83
			1.5T/T2	84	78	65	91	80
Ryoo	2007	128	1.5T	60	94	75	94	86
Sala†	2009	50	1.5T/T1	97	100	100	94	98
			1.5T/T2	91	80	91	80	88
Sanjuan	2008	72	1.0T	71	86	77	83	58
Sato	2009	191	1.5/3.0T	75	86	73	88	83
Savelli	2008	74	N/A	84	81	77	87	82
Sironi	1992	73	0.5T	88	85	68	95	86
Suh	2009	301	N/A	69	74	87	49	59
Torricelli	2008	52	3.0T	93	100	100	92	96
Undurraga	2009	108	1.5T	56	85	79	66	83
Varpula	1993	47	0.02T	83	79	42	96	79

Vasconcelos	2007	101	1.0T	89	100	100	91	95
Yamashita†	1993	40	1.5T/T1	85	96	92	85	85
			1.5T/T2	77	89	77	89	68
Ørtoft	2012	143	1.5T	80	83	80	83	82
Özdemir	2009	64	1.5T	85	79	65	92	81
CT								
Hardesty	2000	25	HCT	83	42	31	89	52
Hasumi	1982	20		100	100	100	100	100
Kim	1995	26		40	75	50	67	62
Tsili	2008	21	MDCT	100	80	94	100	95
Zerbe	2000	54		10	100	100	57	62
PET/CT								
Antonsen	2013	247		93	49	41	95	61

*Results of two reviewers are reported separately. †T1 images were dynamic contrast-enhanced images. CT, computed tomography. HCT, helical CT. MDCT, multidetector CT. MRI, magnetic resonance imaging. NPV, negative predictive value. PET/CT, positron emission tomography/computed tomography. PPV, positive predictive value. T, tesla. T1, T1-weighted images. T2, T2-weighted images.

Preoperative staging by MRI includes the assessment of the inner genitalia, pelvic and para-aortic lymph nodes and abdominal (and thoracic) organs. Accurate staging by imaging methods is problematic, as the pathologic lymph nodes may not be enlarged. According to the literature, the sensitivity, specificity, positive predictive value and negative predictive value of MRI in detecting lymph node metastases or more advanced stage disease (FIGO Stage IIIC–IV) have been 46–100%, 88–99%, 40–86%, 88–100%, respectively (Hori et al. 2009, Hricak et al. 1991, Manfredi et al. 2004, Ortashi et al. 2008, Park et al. 2008, Rockall et al. 2007, Ryoo et al. 2007, Sala et al. 2009, Varpula and Klemi 1993).

7.2.3 Computed tomography

Computed tomography has a limited role in the contemporary preoperative evaluation of endometrial carcinoma. As CT is not accurate enough to depict endometrial pathology inside the uterus, the evaluation of myometrial invasion may be compromised. In the published literature, studies evaluating the performance of computed tomography in the preoperative evaluation of endometrial carcinoma are scarce. In a study comparing CT and MRI, the performance of CT in predicting deep myometrial invasion was inferior to MRI (Kim et al. 1995). However, since the first study by Hasumi and associates (1982), the technical evolution of the scanners has enhanced their performance to a great extent. Modern multidetector scanners have improved the image quality and evaluation of myometrial invasion, with a reported sensitivity, specificity, positive predictive value, negative predictive value and accuracy of up to 100%, 80%, 94%, 100% and 95%, respectively (Table 4). Computed tomography seems to be inferior to MRI in the assessment of lymph node pathology, with a sensitivity, specificity, positive predictive value and negative predictive value of 57%, 69–92%, 31–50% and 81–94%, respectively (Connor et al. 2000, Zerbe et al. 2000).

7.2.4 Positron emission tomography/Computed tomography

Compared to cross-sectional imaging modalities, positron emission tomography (PET) allows for functional imaging of the desired target tissue. PET uses a radiotracer, most commonly 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG), that is accumulated in the cancer cells. The accentuated glucose metabolism of the tumor cells induces the increased uptake of the radiotracer. The areas of the highest concentrations of the tracer can be seen in the PET image as 'hot spots'. The combination of positron emission tomography and computed tomography

(PET/CT) enables the anatomic orientation of the areas of high radiotracer uptake (Iyer et al. 2007). PET can be also be combined with a contrast-enhanced CT (PET/CECT) in order to achieve improved tissue demarcation and diagnostic accuracy (Kitajima et al. 2011b).

In endometrial carcinoma, the use of PET/CT varies according to local practices. It can be used as a diagnostic tool in preoperative planning and in the evaluation of disease recurrence. Despite the almost total absence of comparative studies, the performance of PET/CT is considered to be inferior to MRI in the evaluation of myometrial invasion. In a study by Antonsen and colleagues (2013b), the sensitivity, specificity, positive predictive value and negative predictive value of PET/CT to detect deep myometrial invasion were 92.6%, 48.6%, 40.6% and 94.6%. Even though these performance statistics appear to be comparable to MRI or sonography, in their study the accuracy of PET/CT was the lowest, 60.7% when compared to the accuracies of MRI and transvaginal sonography (65.6% and 71.6%, respectively). However, in the same study, the performance of PET/CT in detecting cervical spread was the best with a sensitivity, specificity, positive predictive value and negative predictive value of 42.9%, 94.3%, 68.6%, and 85.0%. The accuracies of PET/CT, MRI and sonography were 82.7%, 82.3%, and 77.9%, respectively (Tables 3, 4 and 5).

Probably the best value of PET/CT is in the assessment of pathologic lymph nodes, as the increased metabolism exposes these metastatic sites regardless of their size. In the published literature, the sensitivity, specificity, positive predictive value and negative predictive value of PET/CT to detect pathologic lymph nodes has been 43–100%, 91–100%, 43–100%, and 83–97%, respectively. The overall accuracy of the method in lymph node assessment has ranged from 78 to 100% (Antonsen et al. 2013b, Crivellaro et al. 2013, Kitajima et al. 2008, Kitajima et al. 2009, Kitajima et al. 2011b, Kitajima et al. 2013, Park et al. 2008, Picchio et al. 2010, Signorelli et al. 2009, Suga et al. 2011).

Some studies have focused on the assessment of the primary lesion with a hypothesis that high metabolism of the tumor could be a predictor of the aggressiveness of the disease. The metabolic rate is determined by calculating the maximum standardized uptake value (SUVmax) of the region of interest. In the studies by Antonsen et al. (2013c) and Kitajima et al. (2012) the metabolic rate of the endometrial tumor correlated with the stage and grade of the disease. The results of the study by Nakamura and colleagues (2010) are in correlation with the preceding studies, however they did not find a correlation between the SUVmax of the lesion and the stage of the disease.

Table 5. Performances of imaging methods in detecting cervical spread in endometrial carcinoma

Author	Year	Patients	Method	Sensitivity	Specificity	PPV	NPV	Accuracy
2DUS								
Akbayir	2011	298	TV	77	99	87	98	98
Antonsen	2013	209	TV	19	94	55	81	79
Artner	1994	69	TV	67	100	100	95	96
Çelik	2010	64	TV	88	92	79	96	91
Cicinelli	2008	100	TV	53	82	34	91	78
Gabrielli	1996	67	TV	54	87	46	91	82
Kietlinska	1988	45	TV	75	78	25	97	78
Köse	2003	47	TV	75	100	100	98	98
Mascilini	2013	144	D-OCO	73	63	30	91	65
			SA	54	93	64	90	86
Prömpeler	1994	96	TV	71	N/A	N/A	N/A	N/A
Savelli	2008	74	TV	93	92	72	98	92
Ørtoft	2013	156	TV	38	89	43	87	80
3DUS								
Alcázar	2009	107	SA	88	N/A	N/A	N/A	N/A
Jantarasaengaram	2013	60	VCI	100	86	73	100	90
MRI								
Antonsen	2013	226	1.5T	33	95	60	85	82
Hori*	2009	30	3.0T	43/43	100/91	100/60	85/84	87/80
			1.5T	43/43	91/91	60/60	84/84	80/80
Hricak	1987	39	0.5T	100	97	91	100	97
Ortashi	2008	100	1.0/1.5T	19	96	50	86	N/A
Rockall†	2007	96	1.5T/T1	50	90	46	92	85
			1.5T/T2	65	87	57	90	82
Sala†	2009	50	1.5T/T1	88	100	100	98	98
			1.5T/T2	75	100	100	96	96
Sanjuan	2008	72	1.0T	41	97	71	89	46

Savelli	2008	74	N/A	79	87	58	95	85
Seki†	2000	39	1.5T/T1	90	90	75	96	90
			1.5T/T2	80	86	67	93	85
Ørtoft	2013	143	1.5T	54	91	56	90	84
CT								
Hardesty	2001	25	HCT	25	70	14	82	63
Hasumi	1982	20		71	100	100	87	90
Tsili	2008	29	MDCT	78	83	78	83	81
PET/CT								
Antonsen	2013	248		43	94	69	85	83

*Results of two reviewers are reported separately. †T1-weighted images were dynamic contrast-enhanced images. 2DUS, two-dimensional sonography. 3DUS, three-dimensional sonography. CT, computed tomography. D-OCO, distance of outer cervical os to lower margin of the tumor. HCT, helical CT. MDCT, multidetector CT. MRI, magnetic resonance imaging. NPV, negative predictive value. PET/CT, positron emission tomography/computed tomography. PPV, positive predictive value. SA, subjective assessment. T, tesla. T1-weighted images. T2, T2-weighted images. TV, transvaginal. VCI, volume contrast imaging.

At present, the costs and limited availability of PET/CT prevent the routine use of the method in the preoperative assessment of endometrial carcinoma. In the future, functional imaging by positron emission tomography will include a combination with MRI to achieve better soft tissue contrast and enhanced accuracy. However, the development of this application is slow, as there are many technical difficulties involved in the fusion of PET and MRI scanners (Kitajima et al. 2011a).

7.2.5 Histopathologic evaluation

The diagnosis of an endometrial carcinoma is based on the preoperative endometrial biopsy or curettage specimen. Although several studies have investigated the performance of non-invasive imaging modalities to detect endometrial pathology, the simple histopathologic assessment is superior to all other methods. Endometrial biopsy is a simple, office-based method that has proven to be reliable in the detecting of malignant endometrial tumors (Dijkhuizen et al. 2000). The concordance between endometrial biopsy and curettage histology is also reported to be good, 84% (Demirkiran et al. 2012, Fothergill et al. 1992).

The histologic differentiation influences the probability of extrauterine disease. The risk for metastases is 0–7% for well differentiated endometrial carcinomas, and 0–17% for moderately differentiated tumors. Poorly differentiated endometrioid carcinomas have a substantially increased risk for extrauterine disease, 18% (Chan et al. 2008, Chi et al. 2008, Creasman et al. 1987). Type II carcinomas are the most aggressive tumors with a risk for metastases up to 58% (Goff et al. 1994, Sakuragi et al. 2000).

Histologic subtype and differentiation are factors that influence the following decision-making, thus it is of importance that the preoperative and postoperative histologic classifications and grading are the same. However, studies evaluating pre- and postoperative histologic assessments have shown that up to 24% of well differentiated endometrial tumors are upgraded to moderately differentiated or high-grade (poorly differentiated endometrioid carcinoma, papillary serous carcinoma, carcinosarcoma and clear cell carcinoma) cancers in the final histopathological report (Ben-Shachar et al. 2005, Goudge et al. 2004, Lampe et al. 1995, Obermair et al. 1999, Wang et al. 2009). Upgrading may expose the patient to unnecessary adjuvant treatments, as the extent of the primary operation may be inadequate for staging. Sometimes, a re-operation with a complete surgical staging may be warranted.

As type II endometrial carcinoma may arise in an atrophic endometrium, the accuracy of a minute sample in detecting pathology may be questioned. In a study

by Huang and associates (2007), the performance of endometrial biopsy to detect malignancy in patients with a high-grade endometrial tumor in the final histopathologic report was good, with a sensitivity of 99.2%. Regarding only high-risk histology, the concordance with the preoperative and final histology was 85.7%. The result suggests that preoperative endometrial sampling, if achievable, is also sufficient to diagnose endometrial cancer in the atrophic endometrium, and does not need to be verified by dilatation and curettage.

7.2.6 Biomarkers

Blood-derived tumor markers or biomarkers are in clinical use throughout oncology. In gynecology, cancer antigen 125 or carbohydrate antigen 125 (CA125) is the most widely used marker. CA125 is a membrane associated mucin family glycoprotein that is encoded in humans by the MUC16 gene. It is found in many epithelial structures, such as the pleura, pericardium, peritoneum, Fallopian tube, endometrium, and endocervix (Niloff et al. 1984). Since its introduction by Bast and associates (1983) it has been adopted in the diagnosis and follow-up of epithelial ovarian cancer. Niloff and colleagues (1984) were the first to publish on the significance of CA125 in patients with endometrial carcinoma. They found that an elevated level of CA125 correlated with advanced stage. Since then, the role of CA125 has been widely investigated in endometrial carcinoma. It has been reported to correlate with the stage and histologic grade of the disease and to be associated with a poor prognosis (Duk et al. 1986, Hsieh et al. 2002, Jhang et al. 2003, Powell et al. 2005, Rose et al. 1994, Sood et al. 1997, Soper et al. 1990, Yildiz et al. 2012).

However, because of the conflicting results of the published studies, consensus regarding the pathologic cut-off value in the case of endometrial cancer has not been reached, preventing its routine use. Dotters and associates (2000) found that a serum level above 20 IU/mL predicted advanced stage disease in well or moderately differentiated endometrial cancer with a sensitivity of 75%. In a study by Koper and colleagues (1998), the performance of the preoperative CA125 measurement was not as good. When 35 IU/mL was used as a cut-off, only 17% of the patients with metastases were detected. The sensitivity of the test was improved by lowering the cut-off limit to 15 IU/mL, when 53% of advanced stage disesases were detected. Using these limits, the specificity of the test were 95% and 76%, respectively. Kurihara et al. (1998) found that serum CA125 level of 20 IU/mL could predict deep myometrial invasion with a sensitivity, specificity, positive predictive value, and negative predictive value of 69.0%, 74.1%, 58.8%, and 81.6%, respectively. However, in their study, the cut-off limit for the

prediction of metastases was not stated due to considerable overlapping of the CA125 levels between the stages. Lee and colleagues (2010) created a multidisciplinary model for the preoperative evaluation of endometrial carcinoma. They combined preoperative MRI, serum CA125 measurement and preoperative histologic grading to predict the presence of metastases. In the model, a cut-off of 70 IU/mL for serum CA125 was used based on the receiver operating characteristics (ROC) curve analysis. When interpreting the results of a CA125 measurement, one has to take into account that an elevated CA125 level is not necessarily associated with an underlying malignancy, as several other conditions, including pregnancy, endometriosis, liver cirrhosis and even diabetes mellitus may influence the expression of CA125 (Huhtinen et al. 2009, Molina et al. 2012, Turgutalp et al. 2013).

Human epididymis protein 4 (HE4) is a member of the whey-acidic protein (WAP) family and it is encoded by the WAP four-disulfide core domain protein 2 (WFDC2) gene in humans. HE4 was first isolated from the human epididymis (Kirchhoff et al. 1991). As in the reproductive organs, HE4 expression has been found in the oral and nasal mucosa, lung, prostate, and kidney, and its role has been studied in some malignancies, including gastric, lung and breast cancer (Bouchard et al. 2006, Iwahori et al. 2012, Kamei et al. 2010, O'Neal et al. 2012).

The first studies to indicate the relationship between HE4 and ovarian cancer were by Schummer et al. (1999) and Wang et al. (1999). They found through microarray analyses that the HE4 gene is overexpressed in epithelial ovarian cancer. This finding was later confirmed by Hellström and colleagues (2003), who compared the performance of HE4 and CA125 in the detection of ovarian cancer. They stated that HE4 was comparable to or even superior to CA125 as a biomarker for ovarian cancer. In addition, Moore and associates (2008b) found that a combination of these two markers predict ovarian cancer more precisely than either of them alone. Subsequently, they published an algorithm utilizing natural logs of HE4 and CA125 values. With the aid of this algorithm, prediction of an ovarian malignancy was improved with a sensitivity, specificity, positive predictive value, and negative predictive value of 88.7%, 74.7%, 60.1% and 93.9%, respectively (Moore et al. 2009). This algorithm is known as the Risk of ovarian malignancy algorithm (ROMA).

As with every biomarker, HE4 has some weaknesses that must be taken into account in clinical practice. Its concentration in the blood is affected by the subject's age with a positive correlation. The opposite has been observed in pregnant women, whose HE4 levels were found to be lower than that of non-pregnant counterparts (Moore et al. 2011). Although ROMA has separate formulas for pre- and postmenopausal women, it seems that the subject's age has more influence on HE4 concentration than menopausal status itself (Moore et al. 2011).

In endometrial carcinoma, the serum concentration of HE4 has been shown to correlate with the degree of myometrial invasion and the extent of the disease (Angioli et al. 2012, Kalogera et al. 2012, Moore et al. 2008a, Moore et al. 2011, Zanotti et al. 2012). The studies by Bignotti et al. (2011) and Mutz-Dehbalaie et al. (2012) indicated that HE4 has prognostic significance, as its high concentration is associated with a more aggressive type of endometrial carcinoma.

In addition to CA125 and HE4, several other serum markers have been investigated in endometrial carcinoma without significant improvement in the diagnostic or prognostic accuracy. Among these markers are CA153, CA199, CA724, carcinoembryonic antigen (CEA), ovarian cancer antigen X1 (OVX1) and macrophage colony-stimulating factor (M-CSF) (Beck et al. 1997, Hakala et al. 1995, Hareyama et al. 1996, Kanat-Pektas et al. 2010, Olt et al. 1996, Schneider et al. 1999, Yurkovetsky et al. 2007).

7.3 Three-dimensional sonography

In the 1970s, not long after sonography was adopted for clinical use in medicine, the first ideas of producing three-dimensional ultrasound images emerged. The first three-dimensional ultrasound unit was developed by Tom Brown at Edinburgh University, Scotland. The unit, called 'The Multiplanar scanner' consisted of an ultrasound probe equipped with acoustic pulse emitters to track the probe in three-dimensional space. An optical prism was utilized to create a stereoscopic three-dimensional image. The first unit was manufactured in 1975. However, reactions to the new sonographic equipment were not entirely positive. It was considered not to be a useful instrument, and did not succeed commercially (Yagel and Valsky 2008). The precursors of the modern 3D-ultrasound machines were developed in the 1970s by Kretztechnik, Zipf, Austria. These units utilized a mechanical ultrasound probe that consisted of an ultrasound emitter attached to a motorized moving apex. With this setting, the problem of determining the probe orientation in three-dimensional space was solved. However, as with all three-dimensional imaging, three-dimensional sonography necessitated the stacking of images and storing and displaying them was not technically possible until ten years later. Kretztechnic introduced the first commercial 3D-ultrasound unit in 1989: the Combison 330.

3D sonography was first utilized in the imaging of the vascular anatomy of the carotid area. Soon after that, the imaging of the brain, kidneys, prostate, liver and eye was investigated. The two advantages of three-dimensional scanning were promptly noticed: the possibility of an off-line assessment that enabled clinicians to review the acquired images; and more standardized examinations that reduced

operator dependency. In obstetrics and gynecology, the imaging of the fetus by 3D sonography was soon adopted and studied, as the amniotic fluid creates a natural contrast medium for the imaging of the fetal surface anatomy (Rankin et al. 1993). The technical progress of graphics processing units (GPU) and improved computing power enhanced the handling of the three-dimensional information in a manner that eventually produced real-time four-dimensional scanning.

In three-dimensional sonography, the approach for the investigation of ultrasound images differs from two-dimensional imaging. Instead of images, three-dimensional sonographic volumes are acquired. The acquisitions, in theory, constitute of an unlimited number of two-dimensional images within the volume. However, the number of two-dimensional images is in practice limited by the resolution of the ultrasound probe and the display unit. In two-dimensional sonography, the assessed image consists of pixels that have x and y axes. In three-dimensional sonography, the representing unit is a voxel that has three axes: x, y and z. The word 'voxel' is a hybrid of the words 'pixel' and 'volume' and is defined as the smallest distinguishable box-shaped part of a three-dimensional space.

Three-dimensional sonography is often in public opinion comprehended to involve obstetric imaging of the fetal surface anatomy only. In fact, not long after 3D fetal sonography became popular and also extremely profitable worldwide, ACOG announced that a nonmedical or 'recreational' use of ultrasound to acquire two- or three-dimensional fetal images is considered inappropriate because of the potentially harmful but unknown effects of the ultrasound energy on the developing fetus (American College of Obstetricians and Gynecologists Committee on Ethics 2004).

The applications of 3D sonography broaden the spectrum of gynecologic and obstetric sonography from mere assessment of surface anatomy to volumetric and functional evaluation. Three-dimensional acquisition allows for an estimation of volumes, and also an irregular-shaped structure can be assessed and its volume measured given that its margins can be visualized. In addition, combining volumetric assessment with Doppler or power Doppler produces information on the vascularity within the selected volume.

7.3.1 Three-dimensional sonography in gynecologic oncology

The objectives for three-dimensional sonography in gynecologic oncology are the same as with other imaging modalities. 3D sonography is used in the diagnosis, treatment response surveillance, and follow-up of gynecologic malignancies. In pre-treatment evaluation, the main purpose is to assess the extent of the disease in order to design the treatment for an individual patient. Regarding treatment

response evaluation, volumetric assessment by three-dimensional sonography is, in theory, an enticing tool for determining the reduction of the tumor size.

Endometrial cancer

Studies on three-dimensional sonography in the evaluation of endometrial carcinoma predominantly focus on either the measurement of the endometrial tumor volume and its relation to tumor characteristics or on the assessment of myometrial invasion. Tumor volume determination is relatively simple by 3D sonography, when compared to 2D assessment: the volume is extrapolated from several measurements by a dedicated geometric formula. The measurement of the tumor volume seems reasonable, as it has been shown to correlate with the degree of myometrial invasion and the stage of the disease (Chattopadhyay et al. 2013, Mariani et al. 2002, Shah et al. 2005).

The first study to assess the feasibility of three-dimensional sonography in the evaluation of endometrial cancer was by Bonilla-Musoles and colleagues (1997). They published a series of patients with postmenopausal bleeding that were examined by saline-contrast three-dimensional sonohysterography. The number of patients in the study was low, 36 and only three of them had endometrial cancer. However, the diagnostic accuracy in evaluating the endometrium was good with the studied method (100% accuracy regarding EC) and they stated that 3D sonography was a promising utility in the assessment of endometrial pathology.

The reproducibility of the assessment of the endometrial volume by 3D sonography was evaluated by Mercé and associates (2006). The intraobserver reproducibility was considered to be good regarding the evaluation of the endometrial volume with an intraclass correlation coefficient (ICC) of more than 0.97 (95% CI 0.95–1.00). For volume measurement, Mercé et al. used an application called virtual organ computer-aided analysis (VOCAL) that enables a reproducible assessment of three-dimensional volumes. By VOCAL, the operator defines the margins of the selected region of interest (ROI) from a selected plane within the acquired volume, using an automated application or manually. After approximating and drawing the borders, the viewing plane is rotated about a central axis to a defined angle to produce a new plane of view. This procedure is repeated until the full volume of the desired shape is generated (Raine-Fenning et al. 2003a, Raine-Fenning et al. 2003b). The same application was used by Odeh and colleagues (2007) for endometrial volume assessment. They found that endometrial volume of more than 3.56 cm^3, measured by VOCAL, was the best predictor of endometrial carcinoma in women with postmenopausal bleeding, with a sensitivity and specificity of 93.1% and 36.2%, respectively. This finding was also supported

in studies by Mercé et al. (2007) and Yaman et al. (2008), even though their cut-off limits for a pathologic endometrium were different (6.86 cm^3 and 2.7 cm^3, respectively). An opposite result was reported by Opolskiene and colleagues (2010), who found that measurement of the endometrial volume by three-dimensional sonography did not improve the accuracy of diagnosing endometrial cancer when compared to a conventional two-dimensional measurement of the endometrial thickness. They also determined the interobserver reproducibility of the three-dimensional endometrial volume analysis in a saline-contrast sonohysterography. When it was compared to the two-dimensional saline-contrast sonohysterography, no diagnostic improvement was found, and the interobserver reproducibility was in fact better with the two-dimensional scanning (Opolskiene et al. 2009).

Although these pioneering studies attempted to validate the use of three-dimensional sonography in the assessment of endometrial pathology, all of them focused on assessing the diagnostic power of the method to detect cancer preoperatively. Nevertheless, it is unlikely that any imaging modality could overcome the histopathological evaluation of an endometrial biopsy or curettage specimen. Alcázar and colleagues (2009) used 3D sonography to assess the depth of the myometrial invasion in patients that were already known to have endometrial cancer. They utilized the possibility of reviewing the acquired volume in all three orthogonal planes and evaluated the myometrial tumor-free distance to serosa (TDS) for each patient. The TDS correlated with the histological measurement of the unaffected myometrial wall (r=0.649, 95% CI 0.52–0.76). However, the median measured TDS was lower than that measured by a pathologist (7.0 mm vs. 11.0 mm, respectively). With a cut-off of 9.0 mm the sensitivity, specificity, positive predictive value, and negative predictive value of virtual navigation to detect deep myometrial invasion were 100%, 61%, 100% and 50%, respectively. When compared to the subjective assessment of the depth of the myometrial invasion, TDS measurement proved to perform better.

Other objective measurement techniques, both 2D and 3D, were evaluated in a multicenter trial by Mascilini and colleagues (2013). None of the assessed methods, including the measurement of endometrial thickness, tumor/uterine ratio, minimal tumor-free margin, tumor volume and tumor/uterine volume ratio, were found to perform better than a subjective evaluation of the myometrial invasion by two-dimensional sonography. In addition, an objective method to estimate the cervical spread of the tumor was evaluated by determining the distance from the outer cervical os to the lower margin of the tumor. However, when compared to a subjective assessment, no improvement in the diagnostic accuracy was seen.

A recent trial by Jantarasaengaram and associates (2013) introduced a novel method of assessing the myometrial invasion and cervical spread. They used

volume contrast imaging (VCI) display in the assessment of the three-dimensional datasets. VCI is a rendering program that projects multiple slice-shaped volumes on a two-dimensional display. By VCI, the speckling of the sonographic information is reduced, resulting in improved tissue contrast and delineation (Ruano et al. 2004). According to Jantarasaengaram et al., the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the method in detecting deep myometrial invasion were 100%, 89.7%, 78.6%, 100% and 92.5%, respectively. They also found that in the assessment of cervical spread, VCI had a sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of 100%, 86.2%, 73.3%, 100%, and 90.0%, respectively (Jantarasaengaram et al. 2013).

Ovarian cancer

To assess suspected ovarian pathology, morphological evaluation by sonography is often combined with the use of biomarkers. Several predictive models have been developed to characterize ovarian masses, of which probably the most commonly used is the risk of malignancy index (RMI), which utilizes sonographic scoring, serum CA125 measurement and the subject's menopausal status (Jacobs et al. 1990, Kaijser et al. 2013). The evaluation of adnexae is based on similar principles, regardless of the sonographic method, 2D or 3D, used. The typical gray-scale characteristics of a malignant lesion are irregular multilocularity, papillary projections inside a cystic lesion, a solid lesion with irregularity and the presence of ascites in the abdominal cavity. Also, a strong blood flow seen by Doppler sonography may indicate that the assessed lesion is malignant (Kaijser et al. 2013). The results of studies assessing the feasibility of three-dimensional sonography in the evaluation of adnexal masses are conflicting, as some groups have found three-dimensional sonography superior to conventional two-dimensional assessment, while others have found the opposite outcomes (Alcázar et al. 2003b, Alcázar et al. 2007, Alcázar et al. 2012, Bonilla-Musoles et al. 1995, Hata et al. 1999, Kurjak et al. 2000a).

Cervical cancer

Of the three published studies of three-dimensional sonography in the preoperative assessment of cervical cancer, two have focused on tumor volume evaluation and one has described a method for a local staging of cervical carcinoma. Chou and associates (1997) found that tumor volume calculated by 3D

sonography correlated with the volume measured from the postoperative histopathologic specimen. A similar finding was made by Tanaka and Umesaki (2010), who stated that 3D tumor volume correlated with the volume measured by preoperative MRI. In a trial by Ghi and colleagues (2007), the parametrial invasion of the tumor was assessed by three-dimensional sonography. The unique feature of 3D sonography, displaying the rendered image of the coronal plane, was utilized in the study, with a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 100%, 90.0%, 80.0%, 100%, and 92.9%, respectively.

7.4 Three-dimensional power Doppler angiography

Three-dimensional power Doppler angiography (3DPDA) combines the acquisition of three-dimensional volume with power Doppler information to produce quantitative and qualitative data for the vessel distribution and blood flow of the selected tissue. As cancer is dependent on angiogenesis and blood-derived nutrients, the assessment of tumor vascularity by a non-invasive and non-radiating method is attractive. Soon after 3DPDA was introduced by Ohishi and associates (1998) in the assessment of hepatic tumors, it was adopted and evaluated in other fields of oncologic imaging, including gynecologic oncology (Bogers et al. 1999, Kurjak et al. 1998).

7.4.1 Fundamentals of three-dimensional power Doppler angiography

Doppler assessment of blood flow has become an established part of a routine two-dimensional ultrasound examination. The Doppler effect is the shift of the frequency of reflected ultrasound waves from that of the emitted waves. Usually the term Doppler imaging refers to normal or color Doppler, which differs from power Doppler in terms of the technique by which the acoustic information is processed. As ultrasound pulses are transmitted and reflected back from blood cells, two measurable variables are acquired: the frequency change of the emitted and received ultrasound pulses (the Doppler shift) and the amplitude or power of the reflected signal. The first variable describes the sum of the velocity vectors of the blood cells that are parallel to the ultrasound beam. The second variable represents the number of blood cells from which the ultrasound pulse is reflected. The velocity of the flow can be calculated using the formula: $f\Delta\,[\mathrm{Hz}] = 2 \cdot v\,[\mathrm{m/s}] \cdot f_0\,[\mathrm{Hz}] \cdot \cos\Theta / c\,[\mathrm{m/s}]$, where $f\Delta$ is the frequency shift, v is the velocity of the

blood cells, f_0 is the transmitted ultrasound frequency, Θ is the angle between the ultrasound beam and the velocity vector of the flow, and c is the velocity of ultrasound in the blood. As the angle between the assessed flow and the ultrasound beam increases, the sum of the velocity vectors decreases, even though the velocity of the flow remains constant. In addition, when the distance between the probe and the evaluated area of flow increases, the signal intensity is weakened due to the attenuation effect. The signal's attenuation also depends on the frequency of the pulse and the media that the ultrasound pulse is penetrating. The attenuation coefficient α is used to describe the degree of attenuation in decibels (dB) using the formula: attenuation [dB] = α [dB/(MHz · cm)] · depth [cm] · frequency [MHz]. Each tissue has its own attenuation coefficient, thus the attenuation is dependent on the assessed organ (Culjat et al. 2010).

Color Doppler describes the direction and, by quantitating the Doppler shift, the velocity, of the flow. It is not susceptible to signal attenuation to a considerable degree. Nevertheless, some problems may occur when measuring high-velocity flows using color Doppler. When the velocity of the assessed flow is high, the Doppler shift is likewise of high frequency. This necessitates a high rate of collection of the signals, the pulse repetition frequency (PRF). However, a certain amount of time must be allowed for transmitting and receiving the signals. When the Doppler shift frequency exceeds this time interval, a signal artifact called aliasing is seen. Power Doppler measures the intensity, or amplitude, of the echoing signal, regardless of the time interval between the emitted and received pulses. Thus, the direction of the flow is not relevant in power Doppler imaging. However, due to the signal attenuation effect, power Doppler is more susceptible to artifacts caused by tissue characteristics and the distance from the probe to the assessed organ (Anderson and McDicken 2002). The main advantages of power Doppler are that it is sensitive to slow and minute flows and not prone to aliasing.

When a three-dimensional sonographic volume is acquired with power Doppler, the operator can visually evaluate the vascularity within the volume and describe the density and regularity of the vascular tree structure. However, a more quantitative method is to use computer models, of which the histogram facility within the 4DView software (GE Medical systems, Zipf, Austria) is the most commonly used. The operator defines the preferred volume of interest (VOI) by VOCAL, after which the computer program determines the vascularity within the VOI. It is described by three indices that represent different characteristics of the vascularity. The flow index (FI) is a value for the mean intensity of the signal positive power Doppler voxels. The vascularization index (VI) describes the proportion of color-coded voxels within the volume. A combination of these, the vascularization flow index (VFI) represents, in theory, the perfusion in the volume. VFI is formed by multiplying VI and FI and dividing the result by 100. VFI and VI

are given a value between 0 and 100, whereas FI is a percentage (Raine-Fenning et al. 2008a). Although these indices theoretically represent the vascularity of the VOI, actual histologic evidence for this is sparse. Xuan et al. (2007) and Yang et al. (2002) found a correlation between the tissue vascularity detected by three-dimensional power Doppler and the immunohistochemically assessed microvessel density. In contrast, in a recent study by Chen and associates (2012), a correlation was not found between endometrial 3DPDA indices and microvessel density.

Phantom studies have indicated that 3DPDA is sensitive to several confounding factors that must be taken into account when performing it. Ultrasound machine settings have a substantial influence on all three 3DPDA indices, among which the gain and power Doppler power adjustment are the most important. Increasing one or both of these results in higher 3DPDA indices. A change in the PRF will affect the indices with a negative correlation. In addition, the wall motion filter (WMF) adjustment, signal rise and signal persistence must be considered when evaluating 3DPDA indices (Raine-Fenning et al. 2008b). Regarding signal attenuation, a phantom study by Raine-Fenning and colleagues (2008a) indicated that the distance between the probe and the evaluated VOI is inversely correlated with all three 3DPDA indices. The correlation was almost linear in the cases of VI and VFI, and curvilinear with FI, suggesting that the latter may be more resistant to the influence of distance than the other two indices.

The possibility of determining vascular characteristics non-invasively has several potential implications in gynecology, especially in the investigation of infertility and pregnancy. Studies that have assessed endometrial blood flow characteristics suggest that the endometrial 3DPDA indices fluctuate during the menstrual cycle and the diminished perfusion of the endometrium and subendometrium measured by 3DPDA correlate with an adverse pregnancy outcome (Chen et al. 2012, Raine-Fenning et al. 2004a, Raine-Fenning et al. 2004b). Regarding obstetrical applications, Mihu and colleagues (2012) investigated the significance of 3DPDA in the assessment of placental circulation in women with pre-eclampsia. They found that all of the 3DPDA indices were lower in the group with pre-eclampsia, indicating that the diminished placental blood flow due to the increased resistance of the spiral arteries in pre-eclampsia can be assessed by 3DPDA.

7.4.2 Three-dimensional power Doppler angiography in gynecologic oncology

Because of the relatively ambiguous nature of the 3DPDA indices, no cut-off limits for normal or pathologic values have been set. Thus, the results of the published

studies may have demonstrative value only and are difficult implement in clinical practice.

Endometrial cancer

In regard to endometrial carcinoma, the assessment of endometrial vascularity by 3DPDA may have several purposes. It may act as a diagnostic tool, or add to the preoperative risk assessment in the case of a known malignancy. Odeh and colleagues (2007) found that endometrial VI, FI and VFI correlated with the final histology and predicted a pathologic endometrium with a receiver operating characteristics (ROC) area under the curve (AUC) of 0.621, 0.631, and 0.625, respectively. However, the 3DPDA indices did not perform better than endometrial volume assessment using VOCAL. A similar diagnostic approach was assessed by Alcázar and Galván (2009), who stated that the endometrial volume and endometrial vascularity indices correlated with the final histopathological diagnosis, and endometrial VI was the best predictor of endometrial cancer with a ROC area under the curve of 0.902. Opolskiene and associates (2010) evaluated various diagnostic models to predict endometrial cancer in women with postmenopausal bleeding. The logistic regression analysis revealed that a model containing endometrial thickness and endometrial VI had the greatest AUC, 0.86. Using mathematically optimal cut-off values, the sensitivity and specificity of the model to predict an endometrial malignancy were 69% and 86%, respectively. Rossi and colleagues (2012) found that endometrial VI correlated with the histology when diagnosing endometrial cancer by 3DPDA. However, it did not perform better than two-dimensional sonography. The results of a recent study by Makled and associates (2013) are in agreement with the previous studies. Endometrial VI was found to best correlate with the histopathological evaluation. The performed ROC analysis yielded an AUC 0.86 for endometrial VI.

In contrast to the previous trials, a setting with patients that have a recognized cancer may better represent the true clinical situation. In this perspective, Galván et al. (2010) and Mercé et al. (2007) found that the presence of deep myometrial invasion, but not lymph node metastases, was associated with high endometrial vascularity indices.

Ovarian cancer

There are two separate approaches for the assessment of adnexal tumors by three-dimensional power Doppler angiography. The first method involves the subjective

assessment of the power Doppler reconstructed vascular tree. Vascular patterns characteristic of malignant tumors include vessel caliber variability, abnormal branching and tortuosity (Konerding et al. 1999). The subjective evaluation is, however, highly dependent on the examiner's experience and does not improve diagnostic accuracy when compared to two-dimensional gray-scale and color Doppler scanning (Alcázar et al. 2008, Kalmantis et al. 2013, Kupesic and Kurjak 2000, Kurjak et al. 2000b, Laban et al. 2007, Sladkevicius et al. 2007). The other method utilizes the histogram facility, as previously mentioned. It was first introduced in the assessment of ovarian tumors by Alcázar and colleagues (2005). They reported a series of patients with adnexal masses that were evaluated by 3DPDA. Vascularity indices were higher in the group with malignant lesions when compared to patients with benign masses. This finding was supported by the results of subsequent studies using the same technique (Alcázar and Prka 2009, Geomini et al. 2006, Jokubkiene et al. 2007, Kudla et al. 2008, Testa et al. 2005). Nevertheless, the added value of 3DPDA to the diagnosis of ovarian cancer remains unclear, as comparative studies of the method and conventional sonography have shown conflicting results (Alcázar and Rodriguez 2009, Geomini et al. 2007, Kudla and Alcázar 2010, Testa et al. 2005).

Cervical cancer

In the published literature, four studies assess the significance of 3DPDA in the preoperative evaluation of cervical cancer. According to the results of the studies, malignant findings in the histopathological assessment correlate with the 3DPDA indices when compared to normal cervical tissue. However, no correlation between the histological subtypes of cancer, lymphovascular invasion or presence of metastases and 3DPDA indices has been found (Alcázar et al. 2010, Belitsos et al. 2012, Hsu et al. 2004, Testa et al. 2004).

7.5 Angiogenesis and malignant tumors

Tumor growth is dependent on the formation of new blood vessels from pre-existing capillaries, a process called angiogenesis. Angiogenesis is a rate-limiting factor for tumor growth beyond a few millimeters in diameter, as malignant cells are incidental to the supply of oxygen and other nutrients. Normally, angiogenesis is suppressed in adults, with the exception of the activity during female reproductive cycles (ovulation, menstruation, implantation, pregnancy), muscle growth or pathologic processes (wound healing, tumor growth). The importance of

neovascularization was first demonstrated by Gimbrone and colleagues (1972) by implanting cultured tumor cells in the cornea of a rabbit's eye, a site that is avascular by nature. The implanted tumors begun to grow, attracting new blood vessels to the previously avascular area. Folkman and associates (1971) isolated a protein that was responsible for neoangiogenesis in a rat model. It was first called a tumor angiogenesis factor (TAF) and later identified as basic fibroblast growth factor (bFGF) (Shing et al. 1984). The therapeutic possibilities of angiogenic factors were acknowledged soon after their discovery (Folkman 1971). The development of the first antiangiogenic-targeted therapeutic agent, bevacizumab, derived from the finding by Sweeney and colleagues (2001) that the antiangiogenic effect of docetaxel was potentiated by the administration of a recombinant humanized monoclonal antibody to vascular endothelial growth factor (VEGF).

7.5.1 Vascular endothelial growth factor

Vascular endothelial growth factor, or VEGF, is a glycoprotein with a dominant mitogenic activity. The VEGF family consists of five glycoproteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor, or PIGF) that bind to three receptors, VEGFR1 (FLT-1), VEGFR2 (KDR/FLK1) and VEGFR3 (Wu et al. 2010). Other VEGF-related proteins are VEGF-E, which is encoded by the Orf-virus, and VEGF-F, which is expressed in *Vipera lebetina* venom, both binding to VEGFR2 (Gasmi et al. 2002, Shibuya 2006). VEGF-A, often referred to as plain VEGF, is produced in mesenchymal, stromal and epithelial tissues and influences the endothelium through paracrine action. There are several splice variants of the VEGF-A gene: $VEGF_{121}$, $VEGF_{145}$, $VEGF_{148}$, $VEGF_{162}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$ and $VEGF_{206}$, and these isoforms differ in terms of their molecular mass (Wu et al. 2010). Whereas the VEGF family has been demonstrated to be a key component of angiogenesis in general, VEGF-A is agreed to have a particularly important role in tumor angiogenesis (Delli Carpini et al. 2010, Ferrara 1999). Through receptor activation, VEGF promotes vascular proliferation but also regulates vascular permeability, thus it is sometimes referred to as the vascular permeability factor (VPF) (Sitohy et al. 2012).

The receptors of VEGF are membrane-bound tyrosine kinase receptors with similar properties, but their functions are found to be different. FLT-1 and KDR are primarily receptors of VEGF-A and induce angiogenesis, whereas VEGF-C and VEGF-D bind to VEGFR3, activating lymphangiogenesis (Holopainen et al. 2011). The placental growth factor binds principally to VEGFR1. In addition, the soluble form of VEGFR1 (sVEGFR1/sFLT-1) is an antiangiogenic agent which acts by sequestrating free VEGF and thus blocking its action (Wu et al. 2010). The

production of VEGF in a tumor is induced partly directly by the prevailing hypoxic conditions and partly by the hypoxia-inducible factor-1α (HIF-1α) (Carmeliet et al. 1998). In addition, several other oncoproteins such as K-ras, Bcl2, Src, ERBB2, EGFR, FOS and Pttg1 have been found to upregulate the production of VEGF (Kerbel and Folkman 2002). Prognostically, the elevated serum level or immunhistochemical expression of VEGF has been associated with poor outcome in colorectal, pancreatic, hepatocellular, and ovarian carcinomas (Bozas et al. 2010, Chen et al. 1999, Jurgensmeier et al. 2013, Schoenleber et al. 2009, Smith et al. 2011).

The role of VEGF is important in gynecologic oncology, as its monoclonal antibody, bevacizumab, has become a component of the treatment of advanced-stage ovarian carcinoma. Moreover, a recent trial by Zighelboim and associates (2013) showed that patients with recurrent cervical cancer had a survival benefit from bevacizumab as an adjuvant to cytotoxic treatment. However, the role of VEGF in endometrial carcinoma has not yet been established. The results of the immunohistochemical studies on VEGF expression in endometrial carcinoma are not in agreement. Some groups have found a correlation between VEGF expression, poor prognosis, and advanced stage disease, whereas others have not found such an association (Fine et al. 2000, Giatromanolaki et al. 2001, Hirai et al. 2001, Kamat et al. 2007, Ozbudak et al. 2008, Yokoyama et al. 2000, Yokoyama et al. 2003). Regarding preoperative serum levels of VEGF, in a study by Gornall and associates (2001), the serum concentration of VEGF did not correlate with the stage of the disease. Instead, a correlation with the patient's platelet level was observed, suggesting that VEGF measurement has sources of bias that must be apprehended when interpreting the results. A similar finding was made by Dobrzycka and colleagues (2013), who did not find a correlation between VEGF levels and the stage of the disease in type I endometrial carcinoma. However, a positive association was observed in type II carcinomas.

7.5.2 Endoglin

Endoglin (CD105), an accessory protein of the transforming growth factor β (TGF-β) receptor system, is a transmembrane glycoprotein expressed on the cells of an activated endothelium. Endoglin consists of two disulfide-linked subunits of 95 kDa producing a mature 190 kDa homodimeric protein (Barbara et al. 1999). Besides endothelium, endoglin is found in other tissues and cells of the body, including bone marrow, vascular smooth muscle, activated monocytes, differentiated macrophages, the genitourinary tract and embryonic heart (Dallas et al. 2008). A dominantly inherited vascular disorder, hereditary haemorrhagic

teleangiectasia type 1 or Osler-Weber-Rendu disease, is caused by the mutation of the endoglin gene resulting in the development of arteriovenous malformations and mucocutaneous teleangiectases (McAllister et al. 1994). Patients suffer from frequent nose bleeds and hemorrhages in the brain, lungs, and gastrointestinal tract. In endothelial cells, the expression of endoglin is upregulated by hypoxia and TGF-β activation and down-regulated by tumor necrosis factor α (TNF-α) (Dallas et al. 2008, Lebrin et al. 2005, Li et al. 2003). As the function of endoglin is accessory, its role in the activation of cell signaling leading to the initiation of angiogenesis is complex. Several other components are found to participate in the cascade, namely endothelial cell surface receptors ALK1 and ALK5 and intracytoplasmic phosphorylated Smad-proteins (Dallas et al. 2008).

Studies on tumor microvessel density (MVD) suggest that it has prognostic significance in several malignancies, including endometrial carcinoma (Fox et al. 2001, Kaku et al. 1997). As endoglin is highly specific for activated endothelium, it has been used as a marker to immunohistochemically evaluate MVD (Kumar et al. 1999). In the published literature, the expression of endoglin has been associated with poor prognosis in cancers of the gastrointestinal tract, head and neck cancer, breast cancer, prostate cancer, non-small cell lung cancer and brain cancer (Dallas et al. 2008). Regarding malignancies of the female genital tract, endoglin expression or MVD assessed by endoglin has been found to have prognostic significance in ovarian, cervical, and endometrial carcinoma (Erdem et al. 2006, Landt et al. 2011, Saad et al. 2003, Salvesen et al. 2003, Taskiran et al. 2006, Zakrzewski et al. 2011, Zijlmans et al. 2009).

Few studies assess the role of endoglin as a pre-treatment marker in oncology. In a study by Takahashi and colleagues (2001), the high concentration of endoglin in preoperative serum was associated with the presence of metastases in patients with colorectal and breast carcinomas. In addition, Fujita and associates (2009) found that the urine and serum concentration of endoglin was elevated in men with advanced stage prostate carcinoma.

8. Aims of the study

The present study was undertaken to assess the performance of different methods in the preoperative evaluation of endometrial carcinoma. The specific aims of the study were:

1. To investigate the feasibility of three-dimensional power Doppler angiography in the assessment of deep myometrial invasion (I and II).
2. To compare three-dimensional sonography and magnetic resonance imaging in the preoperative assessment of deep myometrial invasion (II).
3. To evaluate the value of preoperative serum HE4 and CA125 concentrations in the prediction of metastases and deep myometrial invasion (III).
4. To assess the significance of markers of angiogenic activity in endometrial carcinoma (IV).

9. Patients and methods

9.1 Patients and study design (I–IV)

One hundred consecutive women diagnosed with endometrial carcinoma and treated at Tampere University Hospital, Finland, between October 2007 and September 2009 were enrolled in this prospective observational study. The criteria for recruitment were a preoperative diagnosis of an endometrial carcinoma based on histopathological evaluation of curettage or endometrial biopsy specimens (I–IV) and eligibility for complete surgical staging (I–IV), transvaginal sonography (I–II) and magnetic resonance imaging (II). The patient demographics and the histopathologic description of the tumors are presented in the Results section (Table 6).

All patients were scheduled for a hysterectomy, bilateral salpingo-oophorectomy, pelvic lymphadenectomy, and peritoneal fluid sampling. Para-aortic lymphadenectomy and infracolic omentectomy were performed when indicated. If histopathological features or preoperative imaging did not indicate a para-aortic lymphadenectomy or omentectomy to be performed, a laparoscopic operation was scheduled. Robot-assisted laparoscopic technique was introduced at the hospital during the last months of the study period, and 15 patients were scheduled for a robot-assisted laparoscopic operation that enables laparoscopic para-aortic lymphadenectomy with omentectomy.

Study II comprised a subset of 20 patients of the total study population that were scheduled for supplemental preoperative magnetic resonance imaging. Studies I and III comprised the same subjects. Study IV included the same population as studies I and III with an exception that type II tumors (papillary serous carcinoma, carcinosarcoma and clear cell carcinoma) were excluded.

9.2 Methods

The results of all assessed methods were correlated with the results of the final histopathological report. The detailed descriptions of the methods are presented in the original communications (I–IV). The off-line assessment of the studied volumes, ELISA analysis and immunohistochemical studies were performed

blinded to the results of the histopathological report. In study II, the ultrasound examination was carried out blinded to the results of the preceding MRI scanning.

All tumors were originally staged according to the FIGO 1988 guidelines, but for the purpose of this study, they were re-staged according to the new FIGO 2009 guidelines (Creasman 1990, Mutch 2009).

9.2.1 Three-dimensional sonography and three-dimensional power Doppler angiography (I–II)

Sonography

All ultrasound examinations were performed on the preceding day or morning of the operation by a Voluson 730 Expert unit (GE Medical Systems, Zipf, Austria) with a multifrequency endovaginal probe (5–9MHz) by one investigator (S.S.). The patients were examined in a supine lithotomy position with an empty bladder. A routine B-mode ultrasound examination was first conducted to assess the pelvic contents in order to allow for an adjustment of the size of the 3-D volume box and the acquisition sweep angle. The three-dimensional power Doppler mode was switched on with settings as follows: frequency, 5 MHz; power Doppler gain, □ 0.6 dB; dynamic range, 20–40 dB; persistence, 2; color map, 5; wall motion filter (WMF), low 1; pulse repetition frequency (PRF), 0.6 KHz; rise, 5; fall, 5. The margins of the volume box were set to cover the contour of the uterus in the sagittal mid-uterine plane. The acquisition sweep angle was set to 75–85 degrees depending on the size of the uterus. Even though pelvic organ movements caused by breathing are minute, to reduce artifacts, the patients were asked to hyperventilate briefly and then hold their breath during the acquisition period, which lasted 15–20 seconds. After a volume was obtained and accepted in terms of quality (entire uterus inside the volume, no movement artifacts or flash signs) it was stored on a hard disk (Sonoview, GE Medical Systems) to be later assessed off-line. Two volumes were stored for each patient.

Three-dimensional power Doppler angiography (I–II)

Off-line assessment was performed by 4DView software version 9.1 (GE Medical Systems), which allows for the visual and quantitative evaluation of the sonographic data on a Windows-based workstation. GPU rendering was switched off before the assessment. The volume was rotated in the necessary dimensions

until a mid-sagittal section was projected in Plane A and a mid-coronal section was seen on Plane C. In study I, the 'Manual' option of virtual organ computer-aided analysis (VOCAL) utility was used to obtain the endometrial volume by outlining the margins of the endometrium inside the volume box in Plane A with 15 degree rotations (Figure 2). With the aid of the histogram facility, the vascular indices VI, FI and VFI were obtained from the selected endometrial volume. After a histogram representing the endometrial blood circulation had been constructed, 5 mm and 10 mm shells were created outside the endometrial volume by use of the 'Edit region of interest' facility (Figure 3). Histograms for these subendometrial shells were obtained in the same manner as for the endometrial volume. Another volume was also selected to cover the body of the uterus by outlining the serous margins of the uterus in Plane A. The uterine volume was constructed using VOCAL with 15 degree rotations. In study II, 5 mm and 10 mm thick shells were created inside this uterine volume, representing the myometrial volumes. Histograms with the respective vascular indices were obtained for these two volumes.

In study I, following the manual sampling, a second automatic analysis was carried out for comparison. This was conducted by selecting the 'Sphere' option in the VOCAL utility. The size of the sphere was set to match the margins of the endometrium in the sagittal section in Plane A. The vascularity indices were obtained from that volume and 5 mm and 10 mm shells surrounding the sphere.

In both studies, the distance between the probe and the endometrium was measured from each volume. The distance was approximated at right angles from the probe's convex surface to the determined center of the endometrium.

Virtual navigation (II)

With the 4DView software, the uterine volume was assessed without power Doppler information by switching off the power Doppler color. The evaluation was initiated by rotating the uterine volume such that a mid-sagittal section was seen in Plane A and a mid-coronal section was projected in Plane C. The endometrium, endomyometrial junction and the depth of supposed invasion were assessed by going through the sagittal and transverse sections in Planes A and B, respectively. A maximal depth of invasion was estimated based on the subjective impression of the examiner. All off-line assessments were performed by one investigator (S.S.).

Figure 2. Schematic illustrations of sonographic sagittal, transverse and coronal sections of the uterus in a three-dimensional dataset. The dark gray color represents the endometrial volume constructed by VOCAL. The dashed lines depict the three orthogonal planes (A, sagittal; B, transverse; C, coronal).

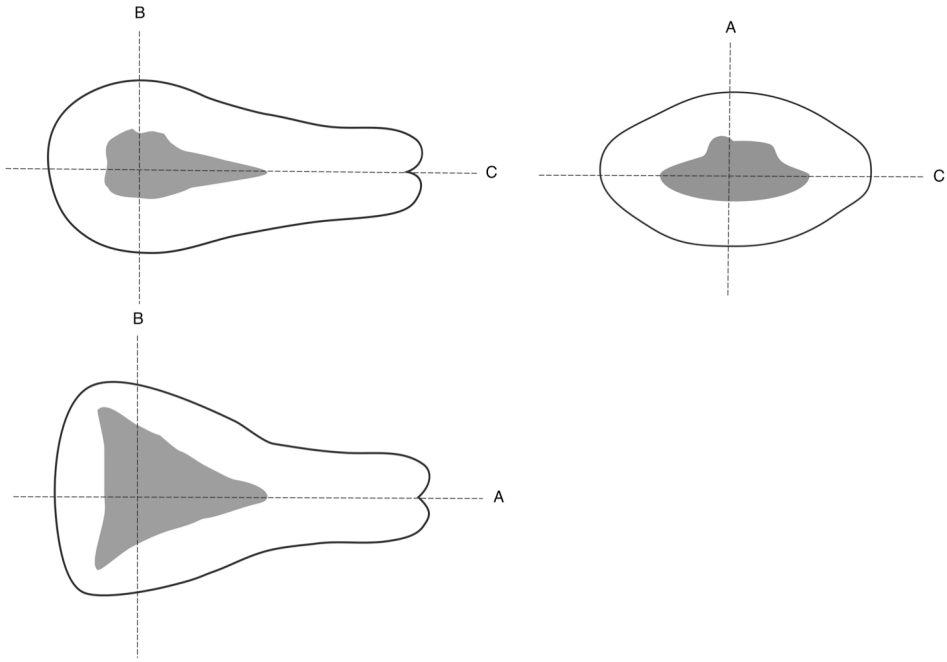


Figure by the author

Figure 3. Schematic illustrations of sonographic sagittal, transverse and coronal sections of the uterus in a three-dimensional dataset. The dark gray color represents the endometrial volume constructed by VOCAL. The light gray color represents a 5 mm shell surrounding the endometrial volume. The dashed lines depict the three orthogonal planes (A, sagittal; B, transverse; C, coronal)

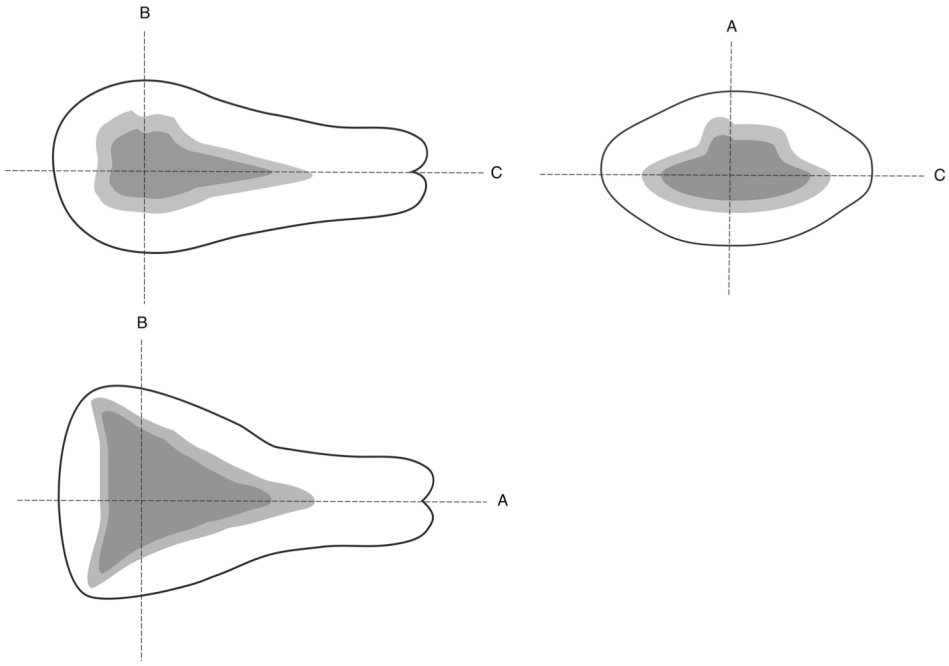


Figure by the author

9.2.2 Magnetic resonance imaging (II)

Magnetic resonance imaging was performed using a Magnetom Trio a Tim System 3 T scanner (Siemens, Erlangen, Germany) with a six-channel Body Matrix coil. The patients fasted for six hours and voided before scanning. Unless contraindicated, twenty milligrams of butyl-scopolamine (Boehringer-Ingelheim, San Cugat del Vallés, Spain) was intravenously administered before the examination to reduce artifacts caused by peristaltic movements. To improve the evaluation of possible cervical invasion, the vagina was filled with Thicken Up Gel® (Milupa GmbH, Fulda, Germany).

In all cases, a coronal turbo spin echo (TSE) T1-weighted image, axial TSE T2-weighted image, parasagittal T2-weighted image, and paracoronal TSE T2-weighted image were acquired. The dynamic MRI scan was performed with a rapid bolus injection of 15 ml of gadolinium-tetraazacyclododecane tetraacetic acid (Gd-

DOTA; Guerbet, Roissy, France) 279.3 mg/ml using the 3D volumetric interpolated breath hold examination (VIBE) sequence. These images were obtained in the paracoronal plane before and 30 and 60 seconds after administration of the contrast medium. A parasagittal fat-suppressed TSE T1-weighted image scan was carried out 120 seconds after contrast injection. MRI scans were assessed as consensus reading by two radiologists experienced in oncological MRI.

9.2.3 HE4 and CA125 measurement in serum samples (III)

The stored serum samples were dissolved and analyzed in two sessions. A total of 98 samples were analyzed, as samples were not available for two patients.

HE4 and CA125 concentrations were measured using commercial ELISA kits (Fujirebio Diagnostics inc., Malvern, PA and Abnova GmbH, Heidelberg, Germany, respectively) according to the manufacturer's instructions. All measurements were performed at room temperature. The plates were read two and five minutes after administration of the stop solution at a wavelength of 450 nm. The manufacturer's reported minimum detection limits for HE4 and CA125 were 15 pM and 5 IU/mL, respectively. A coefficient of variance (CV%) was calculated for the assays. The intra-assay CV% for HE4 was 6.9%, and for CA125 it was 13.6%. A mathematical mean of the results of the duplicate analysis was calculated and used in the statistical analysis.

9.2.4 Endoglin, VEGF and its receptors (IV)

To homogenize the studied population by histology, only women with endometrioid adenocarcinoma were included, yielding a total of 80 samples, as five samples were unavailable for the analysis and in three cases the final histopathological analysis showed no traces of endometrial cancer in the uterus.

Enzyme-linked immunosorbent assay

Human $VEGF_{165}$, sFLT-1 and endoglin (CD105) concentrations were measured at room temperature using commercial ELISA kits (Quantikine®; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. The analysis was carried out as a duplicate and the plates were read two and five minutes after administration of the stop solution. A wavelength of 450 nm was used with

wavelength correction set at 540 nm. The minimum detection limits for VEGF, sFLT-1 and CD105 were 9.0 pg/mL, 3.5 pg/mL, and 7.0 pg/mL, respectively. The intra-assay CV% for VEGF, sFLT-1 and CD105 was 4.2%, 4.5% and 6.0%, respectively. A mathematical mean of the results of the duplicate analysis was calculated and used in the statistical analysis.

Immunohistochemical analysis

All immunostainings were performed on tissue sections obtained from the original formalin-fixed and paraffin-embedded tissue blocks using commercial antibodies (anti-VEGF (A-20), Santa Cruz Biotechnology, Dallas, TX; CD105 antibody Neomarkers, Fremont, CA; VEFR1 antibody, Abcam, Cambridge, UK and VEGFR2 antibody, Cell Signaling Technology, Danvers, MA). The immunostained slides were scanned as virtual slides and viewed by one investigator (S.S.) using Windows-based JVSview software (http://jvsmicroscope.uta.fi). Microvessel density (microvessel count) was assessed from CD105 stained slides by means of click-and-count method. For VEGF, VEGFR1 and VEGFR2, the immunostaining was quantified by evaluating the intensity of the staining and the percentage of stained cells in each specimen. A staining score (no staining; positive staining; strong staining) was calculated from the two assessed variables according to the method described by Yokoyama and colleagues (2000).

9.3 Statistical analysis

In studies I–IV, the distribution of continuous variables was assessed by the Kolmogorov-Smirnov test. Comparison of the groups was evaluated by the Student's t-test or Mann-Whitney U test (I–IV), Kruskal-Wallis test (I, III–IV) and the Fisher's exact test (IV). Correlation was assessed by Spearman's rank correlation (rho) in all studies. In studies I, III, and IV, a ROC analysis was carried out to evaluate the performance of the variables. A comparison of the ROC curves was performed according to the method of DeLong and colleagues (1988). In studies I, III, and IV, a multivariate logistic regression analysis was accomplished. The statistical analyses were performed using SPSS versions 18.0 or 21.0 (IBM Inc, Armonk, NY), with the exception of the ROC curve comparison in study III, which was carried out using MedCalc version 12.0 (MedCalc Software, Mariakerke, Belgium).

9.4 Ethical considerations

The Ethics Committee of Pirkanmaa Hospital District approved the study protocols, and each patient gave their written informed consent for the study.

10. Results

Three of the recruited patients had a final histopathological diagnosis of cancer of a non-uterine body origin (one cervical, one Fallopian tube and one endometrioid ovarian carcinoma). These patients were excluded from the final analysis. The patient demographics and histological diagnoses are presented in Table 6. The diagnostic performance statistics for the assessed methods are presented in Table 9.

10.1 Three-dimensional power Doppler angiography (I)

Endometrial power Doppler signals were identified in 70.1% of the patients (68/97) by the manual and automatic samplings. Subendometrial power Doppler signals in the 5 mm shell were detected in 86 women with manual sampling and in 90 women with the automatic sphere-sampling. 10 mm shell signals were present in all patients with manual sampling and in 94 patients with the automatic method.

10.1.1 Correlation of 3DPDA indices and endometrial volume with deep myometrial invasion and the presence of metastases

The median endometrial power Doppler indices were found to be higher in the group with deep myometrial invasion. The manual and automatic techniques resulted in parallel findings. When the subendometrial shell indices were appraised, a similar tendency was seen. The manually analyzed subendometrial indices were higher in women with a deep invasion, apart from the 10 mm shell FI, which was found not to differ between the two groups. The automatic sphere analysis did not deviate to a great extent from the manual analysis. In the automatic analysis, the flow indices measured from 5 and 10 mm shells did not differ between the groups (Table 7). No correlation between the measured indices and the presence of metastases (≥FIGO Stage IIIC) was found.

Table 6. Patient demographics and histological diagnoses

	Study I	Study II	Study III	Study IV
n*	97	20	95	80
Age (y)	66.9 ± 8.9 (33–87)	68.7 ± 5.6 (59–81)	66.8 ± 8.8 (33–87)	66.9 ± 9.0 (33–87)
Weight (kg)	80.1 ± 16.0 (52–130)	76.9 ± 15.3 (52–119)	80.6 ± 16.3 (52–130)	79.9 ± 15.5 (52–119)
BMI (kg/m²)	30.1 ± 5.8 (20.3–46.1)	28.8 5.2 (20.3–42.2)	30.3 ± 6.0 (20.3–46.1)	30.1 ± 5.8 (20.3–45.7)
Histology (n)				
Endometrioid	88	18	86	80
Grade 1	40	10	40	37
Grade 2	26	4	24	23
Grade 3	22	4	22	20
Serous	4	1	4	0
Mixed cell	2	1	2	0
Clear cell	2	0	2	0
Carcinosarcoma	1	0	1	0
Stage (n)*				
IA	49	8	47	40
IB	28	10	28	25
II	6	0	6	4
IIIA‡	6	1	6	4
IIIB	0	0	0	0
IIIC	6	1	6	6
IVA	0	0	0	0
IVB	2	0	2	1
Myometrial invasion (n)				
<50%	54	8	50	42
≥50%	43	12	45	38

Data are presented as mean ± SD (range) unless otherwise indicated. *Three patients with non-uterine histology excluded. †FIGO 2009 staging. ‡Metastases in the ovaries. BMI, body mass index.

The histologic grading of the tumor did not affect the indices. The median endometrial volume was higher in the group with deep myometrial invasion than in women with no invasion (3.83 cm^3 and 2.12 cm^3, respectively). However, this did not correlate with grade or the presence of metastases. When compared in respect to the presence of myometrial invasion or metastases, the uterine volume did not differ between the compared groups.

The multivariate logistic regression analysis revealed that the endometrial volume and manually acquired endometrial FI were independently associated with the presence of deep myometrial invasion (OR, 1.109; 95% CI, 1.011–1.215 and OR, 1.061; 95% CI, 1.023–1.099. p=0.028 and 0.001, respectively). In the following ROC analysis, the endometrial volume and FI were found to have AUCs of 0.668 (95% CI 0.561–0.776, p=0.004) and 0.730 (95% CI 0.629–0.831, p<0.001), respectively. Using the results of the ROC analysis, the best cut-off value for the endometrial volume was calculated (3.8 cm^3). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the endometrial volume to detect deep myometrial invasion were 51.1%, 71.2%, 60.5%, 62.7%, and 61.9%, respectively. The large number of confounding factors related to 3DPDA assessment prevents the exact evaluation of the diagnostic power of the method. However, as the multivariate analysis suggested that the endometrial FI could predict deep myometrial invasion with some accuracy, the performance statistics were evaluated with a ROC-based cut-off of 26.320. With that cut-off, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy for the detection of deep invasion were 62.2%, 76.9%, 70.0%, 70.2%, and 70.1%, respectively.

10.1.2 The effect of distance on 3DPDA indices

The measured mean distance from the probe to the approximated center of the endometrium was inversely correlated with the 3DPDA indices. There was no difference in the measured distance between the evaluated groups with respect to myometrial invasion or presence of metastases. The patient's BMI had a similar effect on the indices. The measured BMI, the distance from the probe to the endometrium, and the uterine volume were found to positively correlate with each other.

Table 7. Median 3DPDA indices with respect to the presence of deep myometrial invasion

Index	Deep invasion		
	Yes	No	p‡
*Manual**			
Endometrium			
VI	0.917 (0–36.659)	0.009 (0–32.435)	<0.001
FI	28.274 (0–49.346)	18.593 (0–39.325)	<0.001
VFI	0.265 (0–14.986)	0.002 (0–12.755)	<0.001
5 mm shell			
VI	0.742 (0–14.564)	0.093 (0–12.979)	0.001
FI	29.126 (0–42.374)	24.103 (0–40.751)	0.011
VFI	0.189 (0–5.819)	0.023 (0–5.289)	0.002
10 mm shell			
VI	1.117 (0.017–9.937)	0.329 (0.001–7.589)	0.006
FI	33.598 (24.240–43.930)	30.788 (19.310–49.550)	0.057
VFI	0.353 (0.005–4.092)	0.105 (0–3.007)	0.008
Automatic†			
Endometrium			
VI	0.766 (0–38.661)	0.031 (0–32.622)	0.003
FI	26.282 (0–47.105)	18.676 (0–44.151)	0.002
VFI	0.193 (0–16.709)	0.007 (0–14.403)	0.003
5 mm shell			
VI	1.484 (0–26.016)	0.390 (0–28.668)	0.020
FI	30.559 (0–43.667)	28.375 (0–54.078)	0.424
VFI	0.442 (0–10.924)	0.118 (0–11.365	0.026
10 mm shell			
VI	1.378 (0–14.843)	0.633 (0.008–15.639)	0.043
FI	32.517 (0–44.901)	32.759 (20.371–49.143)	1.000
VFI	0.440 (0–6.336)	0.212 (0.002–6.094)	0.050

Data are presented as median (range). *Manual option of VOCAL. †Automatic 'Sphere' option of VOCAL. ‡Mann-Whitney U test. FI, flow index. VFI, vascularization flow index. VI, vascularization index.

10.2 Three-dimensional sonography and magnetic resonance imaging in detecting deep myometrial invasion (II)

Magnetic resonance imaging was found to be more sensitive in the assessment of deep myometrial invasion when compared to sonographic three-dimensional virtual navigation, with a sensitivity of 91.7% and 50.0%, respectively. However, the 3D sonographic evaluation appeared to be more specific (50.0% and 87.5%, respectively). The positive predictive value, negative predictive value, and accuracy

for MRI were 73.3%, 80.0%, and 75.0%, respectively. For 3D virtual navigation, the respective statistics were 85.7%, 53.8%, and 70.0%. A theoretical combination of the methods produced a sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of 100%, 50%, 75.0%, 100%, and 80.0%, respectively. This combination method was calculated by assuming that the diagnostic test is positive if either one or both methods show deep invasion.

The 3DPDA indices measured from the myometrium did not correlate with the degree of invasion. Although the indices were numerically higher in the group with deep invasion, the difference was not statistically significant.

In three sonographic evaluations, the assessment of the endometrium was defective because of uterine fibroids. For these patients, the test result of three-dimensional virtual navigation for the assessment of deep invasion was considered negative as no signs of it were detectable. MRI was found superior in the evaluation of these fibroid uteri.

10.3 Tumor markers in predicting the presence of metastases and deep myometrial invasion (III)

Both CA125 and HE4 were found to be associated with myometrial invasion and metastases (≥FIGO Stage IIIA). All grades included, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy for CA125 to predict a metastatic disease were 28.6%, 96.1%, 57.1%, 88.1%, and 85.7%, respectively. The respective statistics for HE4 were 78.6%, 44.2%, 20.4%, 91.9%, and 49.5%. Regarding deep myometrial invasion, a statistically significant difference in CA125 and HE4 concentrations between the groups was found in grade 1 and grade 3 cancers, but not in moderately differentiated tumors. In the ROC analysis, CA125 had a greater AUC, indicating that it may be superior to HE4 for predicting metastases. However, the difference between the AUCs was not statistically significant. When the actual concentrations of the markers were evaluated, only seven patients had a greater CA125 level than the previously set threshold of 35 IU/mL.

Two possible confounding factors were found to correlate with the evaluated HE4 concentrations. Patient's age and BMI correlated positively with the HE4 level. However, a similar association was not noticed for CA125. When the patient's age and BMI were compared according to the presence of metastases, no correlation was observed.

10.3.1 Risk score analysis

A risk score for the presence of metastases was calculated using the algorithm described by Moore and associates (2009). The logistic regression formula was as follows: predictive index (PI) = -8.09 + 1.04 · ln (HE4) + 0.732 · ln (CA125). A formula for postmenopausal women was used, in spite of there being three premenopausal women in the studied population. Two of these patients were comparable to the rest of the study group by their age (49 and 51 years) and they were included in the analysis. Predicted probability was calculated by the formula: predicted probability (PP) = exp (PI) / [1 + exp (PI)]. A dualistic risk score was then determined using a previously established cut-off of 27.7 for the predicted probability (Moore et al. 2009). By the use of the risk score, the performance of the measured biomarkers in a combination was better than either of them alone. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy for the risk score to predict a metastatic cancer were 71.4%, 89.5%, 55.6%, 94.4%, and 86.7%, respectively.

The multivariate logistic regression analysis revealed that the risk score was the sole independent factor for the presence of metastases (OR, 21.562; 95% CI 5.472–84.963; $p<0.001$). A ROC analysis for the predicted probability produced an AUC of 0.824 for the prediction of extrauterine spread. Based on the ROC curve, a new cut-off limit of 37.0 was determined to perform best in the prediction model. Using this cut-off, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the risk score were 57.1%, 98.7%, 88.9%, 92.6%, and 92.2%, respectively.

10.4 Endoglin, VEGF and its receptors (IV)

The preoperative serum concentration of VEGF was found to be associated with an advanced stage (≥FIGO Stage IIIA). When the histological grades were separately evaluated, the levels of VEGF and sFLT-1 were higher in the metastatic group of the well-differentiated tumors. In moderately differentiated carcinomas, only VEGF was found to differ between the groups. The theoretical active concentration of VEGF, the ratio of VEGF and sFLT-1 was found to correlate with the extrauterine spread of the disease. No association was found between the assessed biomarker levels and histologic grading, deep myometrial invasion or patients' personal features (age and BMI).

The serum concentration of endoglin was not associated with extrauterine spread of the tumor. Furthermore, no correlation was found between the endoglin level and the other histopathological or demographic features.

The multivariate logistic regression analysis indicated that the concentration of VEGF was the single predictive factor for the presence of metastases (OR 1.004, 95% CI 1.002–1.007, p=0.001). The ROC analysis resulted in an AUC of 0.888 (95% CI 0.813–0.964, p<0.001) for VEGF in the prediction of advanced stage. Derived from the ROC curve, a cut-off 707 pg/mL was determined. With that threshold, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of VEGF to predict the presence of metastases were 72.7%, 86.4%, 47.0%, 95.0%, and 88.3%, respectively.

10.4.1 The expression of VEGF and its receptors

The majority of the tumors showed strong staining for VEGF and VEGFR1. In contrast to this, the expression of VEGFR2 was found to be predominantly absent or weak. Probably partly because of this biased outcome, there was no association between the expression of the evaluated markers and the histopathological features of the tumors.

10.4.2 Tumor microvessel density assessed by CD105

The median microvessel density was 30 (range, 3–108; for 20 fields of 40x objective image) in the group with metastases and 40 (range, 5–87) in the group with a localized cancer. The difference was not statistically significant. No difference between the groups was found when the MVD was evaluated with respect to the presence of deep myometrial invasion.

10.4.3 Comparison of serum concentrations and immunohistochemical expression

Although the association between the markers may be theoretical (MVD assessed by CD105 represents the vascularity rather than determining the expression of endoglin; VEGFR1 and sFLT-1 are distinct derivatives of the same receptor), a comparison between the serum levels and tissue expression was accomplished. However, no correlation was found between the investigated markers.

10.5 Correlation of three-dimensional power Doppler angiography with the microvessel density

A statistically significant association was found between the microvessel density assessed by CD105 and manually acquired endometrial VI and VFI. FI did not correlate with MVD (Table 8). The comparison was made for the same population as in study IV. When the immunohistochemical expression of VEGF, VEGFR1 and VEGFR2 were compared to the 3DPDA indices, no association was found.

Table 8. Correlation of the endometrial power Doppler indices with the immunohistochemically assessed tumor microvessel density

	MVD	
Variable	r	p‡
*Manual**		
VI	0.227	0.043
FI	0.205	0.068
VFI	0.236	0.035
Automatic†		
VI	0.154	0.171
FI	0.200	0.076
VFI	0.163	0.149

*Manual option of VOCAL. †Automatic 'Sphere' option of VOCAL.
‡Spearman's rho. FI, flow index. VFI, vascularization flow index.
VI, vascularization index.

Table 9. Performance of the methods in the present study in detecting deep myometrial invasion or presence of metastases

Method	Patients	Endpoint	Sensitivity	Specificity	PPV	NPV	Accuracy
3DPDA							
Endometrial FI	97	MI	62.2	76.9	70.0	70.2	70.1
Endometrial volume	97	MI	51.1	71.2	60.5	62.7	61.9
3DUS							
Virtual navigation	20	MI	50.0	87.5	85.7	53.8	70.0
MRI							
T1/T2	20	MI	91.7	50.0	73.3	80.0	75.0
MRI+3DUS							
T1/T2+VN	20	MI	100	50.0	100	75.0	80.0
Tumor markers							
CA125	91	MET	28.6	96.1	57.1	88.1	85.7
HE4	91	MET	78.6	44.2	20.4	91.9	49.5
Risk score 1*	90	MET	71.4	89.5	55.6	94.4	86.7
Risk score 2†	90	MET	57.1	98.7	88.9	92.6	92.2
Angiogenic markers							
VEGF	77	MET	72.7	86.4	47.0	95.0	88.3

*Cut-off for the risk score 27.7. †Cut-off for the risk score 37.0. 3DPDA, three-dimensional power Doppler angiography. 3DUS, three-dimensional sonography. CA125, cancer antigen 125. HE4, human epididymis protein 4. MET, presence of metastases or extrauterine spread. MI, presence of deep myometrial invasion. MRI, magnetic resonance imaging. NPV, negative predictive value. PPV, positive predictive value. T1, T1-weighted images. T2, T2-weighted images. VEGF, vascular endothelial growth factor.

11. Discussion

The preoperative assessment and targeted treatment of endometrial carcinoma is challenging, and as obesity becomes more common worldwide, the challenge will continue. Furthermore, as operative treatment may be chosen for a growing number of elderly patients, the importance of tailoring the treatment and avoiding unnecessary side effects is accentuated. The general agreement on the surgical treatment of endometrial carcinoma is shifting towards a polarized model. If the patient is considered to have a low risk for extrauterine spread, a hysterectomy with bilateral salpingo-oophorectomy is all that is needed. On the other hand, in the case of an elevated risk for metastases, a complete surgical staging with both pelvic and para-aortic lymphadenectomies is warranted. This emphasizes the value of the preoperative evaluation. Even though minimally invasive techniques are developing and geing adopted in a growing number of institutions, worldwide the majority of operations involving a para-aortic lymphadenectomy are performed via laparotomy. In addition to operation technique-related problems, the post-operative consequences of a lymphadenectomy can be deleterious. The currently available predictive tools, imaging by two-dimensional sonography or cross-sectional methods, and preoperative histology, do not sufficiently assist the operating surgeon in the planning of the treatment. On that account, there is a need for supplementary or more accurate methods for evaluating the risk for extrauterine spread in the individual patient.

In the present study, new preoperative methods utilizing modern imaging technology, tumor markers and markers of angiogenesis were appraised. The endometrial three-dimensional power Doppler indices were found to correlate with the presence of deep invasion. Magnetic resonance imaging was found to perform better than three-dimensional sonography in the evaluation of the degree of myometrial invasion. A novel approach for implementing the risk score analysis in the preoperative evaluation of endometrial carcinoma was introduced. In addition, the preoperative serum level of vascular endothelial growth factor was found to correlate with the presence of metastases. Finally, the microvessel density of the tumor was found to correlate with the quantity of the power Doppler signals within the endometrial volume. Of the evaluated methods, the combination of three-dimensional sonography and MRI was found to be the most accurate for the detection of deep myometrial invasion. Regarding the prediction of extrauterine

spread, the risk score with a cut-off of 37.0 had the best overall accuracy, 92.2% (Table 9). The evaluation of myometrial invasion is just an indirect method to determine the risk for advanced stage disease, the other endpoint of the study. Therefore, a comparison of the evaluated methods in the present study suggests that the direct estimation of the risk using the risk score could theoretically be the most preferable protocol.

11.1 Three-dimensional power Doppler angiography

The role of three-dimensional power Doppler angiography in the preoperative evaluation of endometrial carcinoma is not established. Studies regarding the use of 3DPDA indices as a diagnostic aid suggest that the indices may have some importance (Alcázar and Galván 2009, Makled et al. 2013, Odeh et al. 2007, Opolskiene et al. 2010, Rossi et al. 2012). However, the true diagnostic significance of any imaging method, compared to a simple office-based endometrial biopsy, may be questioned. In that sense, the more interesting application of 3DPDA is in the assessment of patients with a known cancer. In the present study, the endometrial FI was found to best correlate with the presence of deep myometrial invasion. This is in agreement with earlier reports by Galván et al. (2010) and Mercé et al. (2007), although in their studies, intratumoral VI was the best predictor for deep myometrial invasion. The cause of this discrepancy is uncertain, and is likely to simply represent the indefinite nature of the 3DPDA indices.

The assessed subendometrial 3DPDA indices did not appear to perform better than the endometrial indices. There are no comparable studies with which to compare the results of the present study, as the present study was the first to utilize subendometrial shells in this setting. However, the estimation of the endometrial volume by VOCAL is subjective and has potential sources of bias. Thus the capacity of the subendometrial shells to detect myometrial invasion is directly determined by the construction of the endometrial volume. This may have an influence on the results. The significance of endometrial volume measurement in the prediction of deep myometrial invasion is supported by earlier reports by De Smet et al. (2006) and Galván et al. (2010). However, in a recent study by Mascilini et al. (2013), tumor volume measurement by 3D sonography did not prove to be superior to two-dimensional subjective assessment of the invasion.

The manual assessment appeared to perform better than the automatic 'Sphere' option of VOCAL. The virtual spherical sampling of the tumor may well represent the endometrium itself, but the shells surrounding it inevitably contain partly endometrial tissue and partly subendometrial myometrium. The significance of this

confounding factor is yet to be determined. The sphere size itself does not appear to affect the indices, as found by Kudla and Alcázar (2010).

The present study was the first to demonstrate the influence of signal attenuation to the 3DPDA indices in a clinical setting in patients with a gynecological cancer. The distance between the probe and the center of the endometrium affected the 3DPDA indices to a degree that must be taken into account when evaluating the results. The distance was related to the patient's BMI, indicating that obese women have larger uteri, possibly as a consequence of relative hyperestrogenism. At present, there are no methods to compensate the effect of attenuation by any means. However, it is recommended that the distance is acknowledged in successive studies assessing three-dimensional power Doppler angiography. By measuring the distance, the investigators indicate that they are aware of this relevant confounding factor.

There are two options for assessing the acquired 3D volumes off-line. The first option is to use the built-in programs of the sonographic units. The other, and more popular, option is to use the Windows-based 4DView program for the evaluation. However, built-in graphics processing units that enhance the computer's power to work with the graphic content may alter the 3DPDA indices. For that reason, the GPU rendering was switched off during the assessment.

When the formula for the calculation of the attenuation is investigated, the effect is directly proportional to the depth and frequency used. Thus, in theory, a correction factor utilizing the formula could be determined. If the mean distance between the probe and the investigated tissue in a series of assessed patients is called d_0, the attenuation for that given distance is: α [dB/(MHz $\cdot$ cm)] $\cdot$ d_0 [cm] $\cdot$ frequency [MHz]. If the assessed target tissue is the same with all patients and the conditions of the examination (and the machine settings) are standardized, it is likely that the attenuation coefficient α is close to constant. For instance, for soft tissue it is 0.54 (Culjat et al. 2010). When the distance deviating from the mean distance is called d_x, the attenuation for that distance is: α [dB/(MHz $\cdot$ cm)] $\cdot$ d_x [cm] $\cdot$ frequency [MHz]. A correction factor x_{att} for attenuation of ultrasound energy that is reflected from a target that deviates from the mean by its distance could be then calculated using the formula: x_{att} $\cdot$ {α [dB/(MHz $\cdot$ cm)] $\cdot$ d_x [cm] $\cdot$ frequency [MHz]} = α [dB/(MHz $\cdot$ cm)] $\cdot$ d_0 [cm] $\cdot$ frequency [MHz]. From that equation is determined: $x_{att} = d_0/d_x$. This theoretical correction factor is constructed by assuming that power Doppler energy is affected by the distance in a similar manner to ultrasound in general. However, the phantom study by Raine-Fenning and colleagues focusing on the subject demonstrated that in distances up to 5.5 cm in depth, the 3DPDA index FI remained relatively constant. VI and VFI were found to have a linear correlation with the distance, with an approximated reduction of 50% in intensity at a distance of 2.5 cm (Raine-Fenning et al. 2008a).

All of these findings indicate that the problem of signal attenuation in 3DPDA is complex and remains yet to be solved.

In the present study, the number of missing indices was notably high. This may be due to the power Doppler settings used, as the power Doppler gain was set to a low level. On the other hand, it may simply represent the small number of vessels in those evaluated volumes. The attenuation did not seem to explain the missing indices, as the measured distance from the probe to the center of the endometrium was the same in the group with detected vascularity and the group with absent vascularity. This situation has troubled other investigators as well, but not to this extent (Alcázar et al. 2003a, Alcázar and Galván 2009, Galván et al. 2010).

11.2 Magnetic resonance imaging and three-dimensional sonography

Magnetic resonance imaging is globally the primary imaging method to preoperatively evaluate the extent of the disease in endometrial carcinoma. However, the cost and availability of MRI may prevent its routine use. The results of the present study regarding MRI performance are in agreement with the findings of the three earlier reports (Antonsen et al. 2013b, Gordon et al. 1989, Lien et al. 1991). In the majority of the previous studies, the specificity of MRI has been better. The possible causes for the poor specificity were the stretching of the myometrial wall by a large, polypoid tumor and the presence of adenomyosis. Although the studied population was small, only 20 patients, the present study was the first to compare the preoperative significance of three-dimensional virtual navigation and MRI in endometrial carcinoma. The performance of three-dimensional sonography was partly impaired because the three-dimensional volumes were acquired with power Doppler. This results in a poorer resolution and increased susceptibility to movement artifacts. In a previous study by Alcázar and colleagues (2009), the performance of three-dimensional virtual navigation was considered to be good. Although the sensitivity of this sonographic method in the present study was not as good (50% versus 100% for TDS and 92.6% for subjective assessment, respectively) the specificity was better (87.5% versus 61% for TDS and 82.3% for subjective assessment).

A combination of the methods produced an improved performance to detect deep myometrial invasion. According to this model, the more specific three-dimensional sonography could be the primary method of imaging. Patients with a negative result for deep myometrial invasion could be then examined with MRI, which appeared to be more sensitive. Using the combination, the negative predictive value increased up to 100%. However, this protocol may be solely

theoretical, as the proposed sequential imaging using both modalities may be difficult to accomplish in clinical practice.

11.3 The predictive value of CA125 and HE4

The present study was the first to utilize a combination of CA125 and HE4 in the preoperative evaluation of extrauterine spread in endometrial carcinoma. The outcome of the present study implied that the combination of CA125 and HE4 could be beneficial when evaluating the extent of the disease preoperatively. Only one comparative study exists, published recently by Antonsen and colleagues (2013a). They found that the diagnostic power of the markers is improved by combining CA125 and HE4 measurements. A combination of the markers was found to have an AUC of 0.78 for the prediction of metastases. The AUCs for CA125 and HE4 as single markers were 0.77 and 0.70, respectively. Although the present study utilized a different combination formula, a similar difference in the performance of the markers was seen. However, when the AUCs were compared, they did not appear to be statistically significantly different. A comparison of the ROC curves was not performed by Antonsen and colleagues. The added value of the risk score analysis to the preoperative diagnostic process remains to be confirmed, as the number of patients with an extrauterine disease in the present study was rather small, at only 14.

The possible sources of bias regarding the evaluation of CA125 and HE4 in patients with endometrial carcinoma must be recognized. The observed correlation of HE4 levels and BMI deserves further attention. Previous studies have reported no correlation or an inverse correlation between HE4 and BMI (Bignotti et al. 2011, Bolstad et al. 2012). In addition to this, Bignotti and associates (2011) found that smokers had higher concentrations of HE4 compared to non-smokers. One possible explanation for the discrepancy of the results is that smokers tend to have a lower BMI than non-smokers (Sneve and Jorde 2008). However, in the study by Antonsen and colleagues (2013a), an association was not seen between HE4 levels and smoking habits. Regardless of these contradicting findings, the relationship between BMI and endometrial carcinoma must be acknowledged. Patients with endometrial carcinoma are more likely to have high BMI, which may bias the results. Another and more established confounding factor is the age of the subject from which the HE4 concentration is measured. Moore and associates (2011) found that HE4 levels increase with increasing age. In the present study, the logistic regression model to construct the risk score for an extrauterine spread was that determined for postmenopausal women. Two of the three premenopausal women in the studied population were included in the analysis, as their age was

comparable to the rest of the studied cohort. According to Moore et al. (2011), the patient's age has more influence on HE4 levels than menopausal status itself.

11.4 VEGF as a preoperative marker

The serum concentration of VEGF was found to be associated with extrauterine disease when all grades were included. However, the most significant correlation was observed in patients with a well or moderately differentiated carcinoma. Such an association was not found for poorly differentiated carcinomas. From a clinical point of view, the result is not so problematic, as in the case of a poorly differentiated carcinoma, the risk for lymph node metastases is increased as such, ranging from 0 to 28% (Chi et al. 2008). The endpoint in studies III and IV was extrauterine spread, including patients with metastases in the ovaries (FIGO Stage IIIA). This endpoint was chosen as it presumably represents the metastatic potential of the tumor. Thus, a greater number of patients were included in the pathologic group, adding to the diagnostic power of the analysis. However, this may be confusing when comparing the outcome of the present study with other reported results that may have different endpoints.

In the two previous studies assessing the significance of the preoperative serum level of VEGF in endometrial carcinoma, an association was not observed between VEGF and the stage of the disease in endometrioid carcinoma (Dobrzycka et al. 2013, Gornall et al. 2001). In the other study, in a subgroup of type II carcinomas the FIGO stage was found to have a positive correlation with serum VEGF concentration (Dobrzycka et al. 2013). The other study group detected patients' platelet levels to be associated with the observed VEGF concentrations (Gornall et al. 2001). A similar comparison with patient's platelet levels was not made in the present study. Regarding the study by Gornall and colleagues (2001), the discrepancy in the outcome, compared to the present study may be the result of the difference in the size of the study populations (80 versus 37, respectively). The studied population assessed by Dobrzycka and associates (2013) consisted of 70 patients with type I carcinoma of which only 22 were surgically staged. This relatively high proportion of non-staged cases may explain the contrasting result.

The multivariate analysis revealed that serum VEGF concentration was the sole independent factor for the presence of metastases (OR, 1.004, 95% CI, 1.002–1.007, $p=0.001$). Nevertheless, the odds ratio is relatively low and thus the true clinical significance of the multivariate analysis is questionable.

The biological effective concentration of vascular endothelial growth factor, the VEGF/sFLT-1 ratio, was found to correlate with the presence of extrauterine disease. The significance of sFLT-1 has been evaluated in several malignant

conditions, including breast cancer, pancreatic cancer, and acute myeloid leukemia, with an observed association with poor prognosis (Aref et al. 2005, Bando et al. 2005, Chang et al. 2008). The VEGF/sFLT-1 ratio has been found to have prognostic significance in breast and pancreatic cancer (Bando et al. 2005, Chang et al. 2008). As regards endometrial carcinoma, there are no previous studies for comparison.

11.5 Correlation of three-dimensional power Doppler angiography with the tumor microvessel density

In the present study, the tumor microvessel density was found to be associated with endometrial manually analyzed VI and VFI. In theory, VI represents the amount of vessels, as it determines the proportion of color-coded voxels within the selected volume. In addition, VFI is proportional to VI. In that sense, the correlation with MVD seems reasonable. However, certain inaccuracies must be considered. The microvessel density was assessed from a microscopical section of the endometrium. In contrast, the 3DPDA indices were acquired from the total endometrial volume. This discrepancy may bias the results. In addition, the used antibody binds to tumoral endothelial cells with a high affinity. Thus, cell clusters without a lumen are also stained and included in the microvessel count. In this respect, the microvessel density does not necessarily represent the number of vessels with a blood flow.

Only four previous studies have focused on this significant question regarding 3DPDA. In a study by Fleischer and associates (1999), the immunohistochemically evaluated microvessel density of a transplanted murine tumor correlated with the three-dimensional power Doppler assessment. Similarly, Xuan and colleagues (2007) observed the immunohistochemically assessed microvessel density in a genetically engineered mouse prostate cancer to be associated with the vascularity assessed by three-dimensional power Doppler sonography. Yang and associates (2002) observed a respective correlation in breast cancer. However, the ultrasound units in these studies were different to the one used in the present study, and the 3D power Doppler information was not expressed as 3DPDA indices. The only comparable study is by Chen et al. (2012), who found that endometrial microvessel density assessed by immunhistochemistry did not correlate with the 3DPDA indices. Nevertheless, the tissue samples were taken from endometrial biopsies that presumably only partly represent the true vasculature of the endometrium.

The outcome of the present study supports the hypothesis that the vascularization index VI represents the amount of the vessels within the observed volume of interest.

11.6 Considerations for future research

Probably it is not possible to overcome every confounding factor of 3DPDA when used in a clinical setting. Therefore, the potential clinical applications of 3DPDA may prove to be difficult to implement in clinical practice. In the present study, 3DPDA did not improve the detection of deep myometrial invasion compared to the general understanding of the accuracy of the present conventional sonographic methods. However, three-dimensional sonography may not be entirely unsignificant. The present study and the work by Alcázar and colleagues (2009) are the only trials to determine the value of three-dimensional virtual navigation in the preoperative evaluation of endometrial carcinoma. This approach, as an alternative to two-dimensional sonography, deserves further attention in the subsequent studies. In addition, the added value of the contrast agents and saline instillation should be determined.

The relevance of the HE4 measurement in endometrial carcinoma should be verified in larger studies, also including the evaluation of its relation to BMI. If the association between BMI and HE4 levels is found to be clinically relevant and linear, it will be possible to construct a correction formula to compensate for the influence of BMI.

Regarding angiogenesis, the biologically active proportion of VEGF, the VEGF/sFLT-1 ratio, warrants more investigation. However, it must be acknowledged that the initiation of angiogenesis has several pathways, and the VEGF pathway is just one of them. VEGF is also an enticing target for treatment, and the results of the studies assessing the significance of the anti-VEGF antibodies in the treatment of endometrial carcinoma are highly anticipated.

12. Summary and conclusions

The present study was undertaken in order to evaluate the significance of three-dimensional angiography, three-dimensional sonography, and magnetic resonance imaging in the preoperative assessment of endometrial carcinoma. In addition, blood-derived tumor markers were investigated in the preoperative prediction of extrauterine disease. A novel method utilizing serum CA125 and HE4 measurements was examined. The angiogenic activity of endometrial carcinoma was assessed by enzyme-linked immunosorbent assay and immunohistochemistry. Possible confounding factors regarding the utilized imaging modalities and the tumor markers were observed. Finally, the association between the immunohistochemically determined microvessel density and the three-dimensional power Doppler indices was evaluated.

The main findings and conclusions of the study were:

1. Endometrial FI and endometrial volume assessed by three-dimensional power Doppler angiography correlate with the presence of deep myometrial invasion. The possible confounding effect of distance is difficult to overcome in a clinical setting.
2. Magnetic resonance imaging is more sensitive than three-dimensional sonography in the assessment of deep myometrial invasion. A sequential assessment by 3D sonography and MRI may be recommended given that the resources of the institution are sufficient.
3. The combination of CA125 and HE4, the risk score, is a better predictor of an extrauterine disease than either of the markers as a single predictive factor. However, the influence of the patient's BMI on the HE4 level remains as yet unanswered.
4. The preoperative serum level of VEGF correlates with the presence of metastases. Nevertheless, VEGF measurement has sources of bias that must be acknowledged. The VEGF/sFLT-1 ratio may act as a derivative for the biologic activity of VEGF.
5. The tumor microvessel density assessed by immunohistochemistry correlates with the degree of the vascularization determined by three-dimensional power Doppler angiography.

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Preoperative assessment of endometrial carcinoma by three-dimensional power Doppler angiography

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KEYWORDS: endometrial cancer; power Doppler; three-dimensional ultrasound

ABSTRACT

Objective *Preoperative evaluation of the depth of myometrial invasion in endometrial carcinoma is challenging. The objective of this study was to evaluate the usefulness of three-dimensional power Doppler angiography (3D-PDA) in this setting.*

Methods *Sonographic and histological data on 100 consecutive cases of endometrial carcinoma were analyzed. The endometrial and myometrial vascular indices VI (vascularization index), FI (flow index) and VFI (vascularization flow index) were calculated by 3D-PDA. The results were compared with a complete surgical staging.*

Results *The mean (± SD) age of patients was 67.1 ± 8.8 (range, 33–87) years. Forty-six patients had deep (≥ 50%) myometrial invasion. Eight patients had metastases, seven of them with deep invasion. Three patients were found to have carcinomas of non-uterine origin on histology, and these were excluded from further statistical analysis. The median endometrial and myometrial vascular indices were higher in the group with deep invasion than in the group without. Following multivariable analysis of the indices only the endometrial FI was independently associated with deep invasion (OR, 1.061; 95% CI, 1.023–1.099;* $P = 0.001$*). However, a greater endometrial volume was also an independent predictor of deep invasion (OR, 1.109; 95% CI, 1.011–1.215;* $P = 0.028$*).*

Conclusion *Our study suggests that endometrial and, to a lesser degree, myometrial vascular indices and endometrial volume correlate with the depth of myometrial invasion in endometrial carcinoma. Copyright © 2012 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Endometrial carcinoma is the most common form of gynecologic cancer, and it is the fourth most common malignant tumor among women worldwide[1]. Endometrial carcinoma generally has a favorable prognosis, since most cases (75%) present at an early stage of the disease[2].

Surgery is the cornerstone of treatment. Complete surgical staging includes hysterectomy and bilateral salpingo-oophorectomy, pelvic (and para-aortic) lymphadenectomy and peritoneal fluid sampling or lavage for cytology. However, the need for a lymphadenectomy has been questioned if the risk of metastasis is low, i.e. in the case of low-grade carcinomas[3]. Preoperative evaluation of the risk of metastasis is based on histological analysis of endometrial biopsy or curettage specimens and assessment of tumor invasion in the myometrium by imaging methods.

The depth of myometrial invasion is an independent prognostic factor in endometrial carcinoma, with an increased risk of metastasis when the invasion is ≥ 50% of the myometrial thickness[4]. The sensitivity and specificity of transvaginal two-dimensional (2D) ultrasonography in detecting deep invasion ranges from 79%–87% to 75%–100%, respectively[5–7]. Recently, application of three-dimensional (3D) ultrasound has been shown to have promising results in detecting deep invasion in patients with endometrial carcinoma, with a sensitivity of 100% and a specificity of 61%[8]. Magnetic resonance imaging (MRI) is purportedly the most accurate method of assessment of myometrial invasion, with a sensitivity of 50%–93% and a specificity of 82%–100%[9–14]. However, the cost and often limited availability can restrict the routine use of MRI in this setting.

The combination of 3D-ultrasound and power Doppler, or 3D-power Doppler angiography (3D-PDA), allows for an objective assessment of vasculature and volume

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calculation in irregular-shaped structures. As a method, 3D-PDA has been proven to be reproducible[15–17]. Several groups have studied the performance of 3D-PDA in gynecological oncology, but so far its clinical value remains unclear.

The aim of this study was to evaluate the performance of 3D-PDA in detecting the presence of deep invasion and predicting metastasis in patients with endometrial cancer.

METHODS

One hundred consecutive patients presenting with endometrial carcinoma at the Department of Obstetrics and Gynecology of Tampere University Hospital between October 2007 and September 2009 were enrolled in this prospective observational study. All patients gave written informed consent, and the study protocol was approved by the Ethics Committee of Pirkanmaa Hospital District. The patients were surgically staged according to the FIGO 2009 recommendations[18]. Ultrasound examination was performed 24 hours prior to surgery.

All ultrasound examinations were performed by one of the authors (S.K.S.) using a Voluson 730 Expert unit (GE Medical Systems, Zipf, Austria) with a multifrequency endovaginal probe (5–9 MHz). All patients were scanned in the lithotomy position after emptying the bladder. After a routine B-mode evaluation, 3D-power Doppler was used to assess endometrial and myometrial vascularization. Power Doppler settings were as follows: frequency, 5 MHz; power Doppler gain, −0.6 dB; dynamic range, 20–40 dB; persistence, 2; color map, 5; wall motion filter (WMF), low 1; pulse repetition frequency (PRF), 0.6 KHz; rise, 5; fall, 5. The acquisition sweep angle was set to 75–85° depending on the size of the uterus.

To reduce artifacts caused by movement, the patients were asked to hyperventilate briefly and then hold their breath during the volume acquisition. A 3D volume containing the uterus was then obtained. The volume acquisition time varied between 15 and 20 seconds depending on the size of the volume box. The patients did not report any problems with holding their breath for such a short time. Once a 3D volume was obtained, it was briefly analyzed and, if it met the requirements (no artifacts caused by movement, whole uterus inside the volume box), it was stored on a hard disk (Sonoview, GE Medical Systems). Two volumes were obtained from each patient.

The stored volumes were later analyzed by the same investigator (S.K.S.) using the 4DView software (GE Medical Systems, v 9.1). First, the volume was rotated until sagittal, transverse and coronal sections of the uterus corresponded with Planes A, B and C, respectively. Using the 'Manual' option of Virtual Organ Computer-aided AnaLysis (VOCAL), the endometrium was outlined inside the volume box with 15° rotations in Plane A (Figure 1a). Using the histogram facility, vascularization index (VI), flow index (FI) and vascularization flow index (VFI) were calculated from the selected endometrial volume. VI is a measurement of the number of color-coded voxels in the selected volume and is expressed as percentage. FI is a measurement of the mean power Doppler intensity in the color-coded voxels and is expressed as a value ranging from 0 to 100. VFI is a combination of VI and FI and is also expressed as a value ranging from 0 to 100. Selection of the endometrial volume was done by following the margins of the endometrium, excluding the suspected areas of deep invasion in the myometrium. Subsequently, 5-mm and 10-mm shells representing the myometrium were created outside the endometrial volume using the automatic 'Edit region of interest' option and the vascular indices were calculated from these volumes, respectively (Figure 1b). The 'Manual' option of VOCAL was also used to estimate the volume of the uterus by outlining the serous margins of the uterus from the cervicoisthmic region towards the fundus with 15° rotations in Plane A. For comparison, a second analysis was carried out using automatic sphere sampling from the other 3D volume that had been stored. This was done by selecting the 'Sphere' option of VOCAL. The size of the sphere was manually set to match the margins of the thickest part of the endometrium in Plane

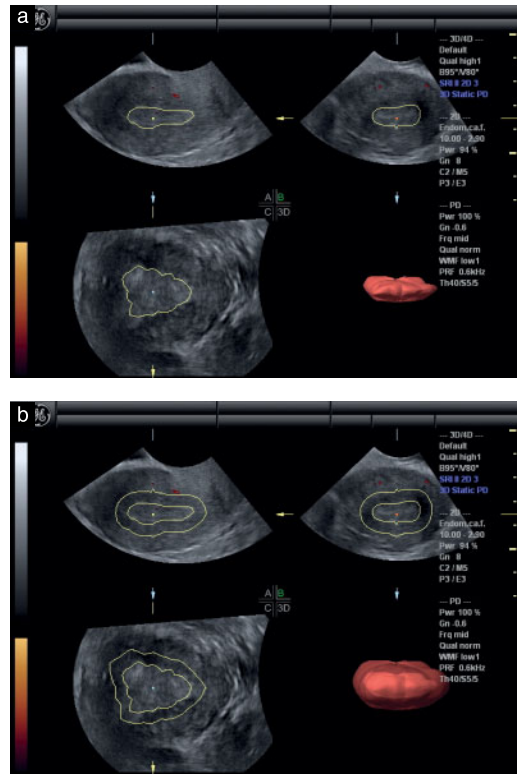

Figure 1 (a) Using the 'manual' option of VOCAL, the endometrium was outlined inside the volume box with 15° rotations in Plane A. (b) A 5-mm shell surrounding the endometrium was subsequently created around the outside of the endometrial volume; this was also repeated with a 10-mm shell (not shown).

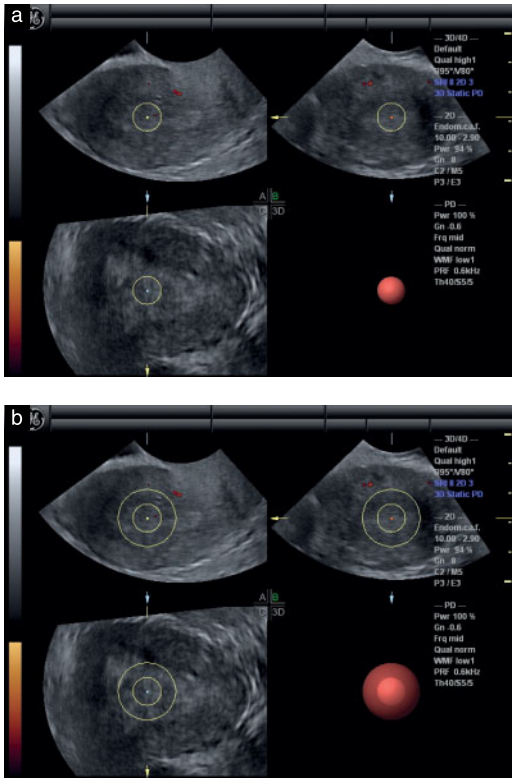

Figure 2 (a) Using the 'automatic' option of VOCAL, the size of the sampling sphere was set to match the thickest part of the endometrium. (b) A 5-mm shell surrounding the sphere was subsequently created; this was also repeated with a 10-mm shell (not shown).

A (Figure 2a). The vascular indices were calculated from that volume and 5-mm and 10-mm shells surrounding it, respectively (Figure 2b). The distance between the probe and the endometrium was measured for each patient at a right angle from the probe's convex surface to the approximate center of the endometrium.

The ultrasound examination and the vascularization analysis were performed blinded to the results of the preoperative endometrial biopsy or curettage specimen analysis and final histopathological report; however, the examiner was aware that the patients had endometrial cancer.

Statistical analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to evaluate normal distribution of continuous variables. Comparison of the groups was performed using Student's *t*-test, Mann–Whitney *U*-test and Kruskal–Wallis test as appropriate. Correlation was evaluated using Spearman's correlation coefficient. Variables that were statistically significant on univariable analysis were compared by a multivariable logistic regression analysis.

RESULTS

The mean (± SD) age of the patients was 67.1 ± 8.8 (range, 33–87) years. Patient demographics, along with histological diagnosis, are presented in Table 1. Ninety-seven patients had uterine cancer, of whom 88 had an endometrioid histology. The 'other' group in Table 1 includes three tumors preoperatively diagnosed as endometrioid adenocarcinomas, but which were subsequently reclassified as a cervical, Fallopian tube and an endometrioid ovarian carcinoma, respectively, based on the final histopathological report. These cases were excluded from the statistical analysis.

Lymphadenectomy as planned was performed in 95 patients. In five cases, the surgeon refrained from performing a lymphadenectomy because of patient obesity. One of these patients had a final diagnosis of Fallopian tube carcinoma. Cases with unknown lymph node status were excluded from the statistical analysis for comparisons according to the presence or absence of metastasis.

Histopathological examination of the hysterectomy specimen showed a deep myometrial invasion in 45 patients, whose endometrial volume was significantly

Table 1 Patient characteristics and histological diagnosis ($n = 100$)

Characteristic	*Value*
Age (years)	67.1 ± 8.8 (33–87)
Weight (kg)	80.2 ± 16.2 (52–130)
BMI (kg/m^2)	30.1 ± 6.0 (18.3–46.1)
Histology	
Endometrioid adenocarcinoma	88
Grade 1	40
Grade 2	26
Grade 3	22
Serous adenocarcinoma	4
Mixed cell	2
Clear cell	2
Carcinosarcoma	1
Other	3
Stage*	
IA	49
IB	28
II	6
IIIA	6
IIIB	0
IIIC	6
IVA	0
IVB	2
Myometrial invasion	
< 50%	54
≥ 50%	46
Leiomyomas	9
Personal history of malignancy†	7
Personal history of HRT	30
Estrogen + progestin	26
Estrogen only	1
Not known	3
Mean duration of HRT (years)	8.2 ± 8.5 (1–30)

Data are presented as mean ± SD (range) or *n*. *FIGO 2009 classification[18], three patients with non-uterine histology excluded. †Breast cancer. BMI, body mass index; HRT, hormone replacement therapy.

Table 2 Endometrial volume with respect to tumor characteristics

Characteristic	n	*Endometrial volume (cm³, median (range))*	P
Deep invasion			0.004*
Yes	45	3.83 (0.51–40.38)	
No	52	2.12 (0.25–11.01)	
Metastases			0.075*
Yes	8	5.19 (1.54–33.66)	
No	85	2.64 (0.25–40.38)	
Tumor grade			0.096†
1	40	2.71 (0.64–11.01)	
2	26	1.89 (0.41–40.38)	
3‡	31	4.16 (0.25–37.73)	

*Mann–Whitney *U*-test. †Kruskal–Wallis test. ‡Including serous adenocarcinoma, mixed cell carcinoma, clear cell carcinoma and carcinosarcoma.

greater than that of the 52 patients without deep invasion (Table 2). Fourteen patients had an extrauterine spread of the disease (≥ FIGO 2009 Stage IIIA) and the majority ($n = 11$) of them also had a deep tumor invasion in the myometrium. Of the eight patients with lymph node or distant metastases, one patient did not have deep myometrial invasion. The presence of metastases and an increasing tumor grade also tended to be associated with an increasing endometrial volume, but not significantly (Table 2).

The median vascular indices using each technique are presented in Table 3. Endometrial power Doppler signals were identified in 68 of 97 patients using both manual tracing and automatic sphere-sampling. Power Doppler signals were identified in the 5-mm shell in 86 patients using the manual analysis and in 90 using the automatic 'Sphere' analysis. Power Doppler signals were identified in the 10-mm shell in 97 and 94 patients using manual and automatic analysis, respectively. If power Doppler signals were not detected, vascular indices VI, FI and VFI were all given a value of 0. For all patients, endometrial vascular indices tended to be smaller than those in the 5-mm shell which, in turn, tended to be smaller than those in the 10-mm shell. Thus, in no instance was vascularity detected in the endometrium while being absent in the shells surrounding it. Of the patients with no detectable vascularity, 26 had an endometrioid histology, two had a serous adenocarcinoma and one had a clear cell carcinoma. As for the tumors, 26 were confined to the uterus, and three were advanced stage (≥ Stage IIIA) tumors. Six patients also had a deep myometrial invasion.

The mean (± SD) distance between the surface of the probe and the center of the endometrium was 2.78 ± 0.86 cm. In the group with no deep invasion the mean distance was 2.59 ± 0.60 cm and in the group with deep invasion it was 3.01 ± 1.06 cm. The difference was not statistically significant. We separately analyzed the mean distance in the cases where endometrial power Doppler signals were not detected and where they were, and it was 2.73 ± 0.88 cm and 2.81 ± 0.86 cm, respectively ($P > 0.05$). Vascular indices were negatively correlated with body mass index (BMI) and distance between the probe and the center of the endometrium, this negative correlation being statistically significant in some cases (Table 4). BMI correlated with the distance to the center of the endometrium and the volume of the uterus (correlation coefficient, 0.370 and 0.339; $P = 0.001$ and 0.001, respectively (Spearman's rho)) but not with the degree of myometrial invasion, histological grade or the FIGO stage of the disease. The mean (± SD) volume of the uterus was 71.55 ± 54.83 cm³. There was no statistically significant difference in the mean uterine volume between the groups when compared according to the presence of deep myometrial invasion or metastases.

In the multivariable analysis only the endometrial volume and the manually analyzed endometrial FI were independently associated with deep invasion (OR, 1.109 and 1.061; 95% CI, 1.011–1.215 and 1.023–1.099; $P = 0.028$ and 0.001, respectively (forward stepwise logistic regression analysis)). In comparison, we performed a second multivariable analysis in which the patients who did not have detectable endometrial vascularity were excluded. The second analysis left manually analyzed endometrial FI as the sole independent factor associated with deep invasion (OR, 1.094; 95% CI, 1.012–1.182; $P = 0.024$ (forward stepwise logistic regression analysis)).

DISCUSSION

To our knowledge, this is the first study to evaluate myometrial vascularity by 3D-PDA with respect to the presence of deep myometrial invasion and/or metastases. We found that endometrial vascular indices best correlated with the presence of deep myometrial invasion, rather than the indices measured from the surrounding shells that represent the subendometrial myometrium. Consequently, it seems that evaluation of the vascular characteristics of the endometrium, rather than of the myometrium, is important in assessing the risk of deep myometrial invasion. Thus, subsequent studies should concentrate on the endometrial vasculature, taking into account the studies of Mercé *et al.* and Galván *et al.* where endometrial vascular indices correlated with the presence of deep myometrial invasion[19,20].

In a relatively high number of patients vascular indices were detected in neither the endometrium nor in the shells surrounding it. The problem of missing indices has affected other studies also, albeit to a lesser extent[20–22]. Based on the results of phantom studies, it is known that the distance between the probe and the target tissue has an influence on vascular indices[23,24]. However, signal attenuation does not seem to explain the missing power Doppler signals, since there was no difference in the distance between the group where signals were detected and that where they were not. Missing signals may have been due to the power Doppler settings used; in order to reduce the number of false signals, power Doppler gain was set to a relatively low level (−0.6 dB). We did not exclude these patients from the statistical analysis, since the lack of signals presumably reflects a lesser amount of vascularity as compared to the group where vascularity

Table 3 Median vascular indices with respect to presence of deep invasion and presence of metastases in the final histopathological report

		*Deep invasion**				*Metastases†*		
Index	n‡	*Yes* (n = *45*)	*No* (n = *52*)	P§	n‡	*Yes* (n = *8*)	*No* (n = *85*)	P§
Manual¶								
Endometrium	68				66			
VI		0.917 (0–36.659)	0.009 (0–32.435)	< 0.001		1.176 (0–18.942)	0.142 (0–36.659)	0.244
FI		28.274 (0–49.346)	18.593 (0–39.325)	< 0.001		22.035 (0–37.407)	22.964 (0–49.346)	0.382
VFI		0.265 (0–14.986)	0.002 (0–12.755)	< 0.001		0.261 (0–7.086)	0.033 (0–14.986)	0.269
5-mm shell	86				84			
VI		0.742 (0–14.564)	0.093 (0–12.979)	0.001		1.147 (0.002–8.996)	0.273 (0–14.564)	0.150
FI		29.126 (0–42.374)	24.103 (0–40.751)	0.011		30.613 (19.739–40.523)	25.557 (0–42.374)	0.317
VFI		0.189 (0–5.819)	0.023 (0–5.289)	0.002		0.340 (0–3.121)	0.080 (0–5.819)	0.172
10-mm shell	97				93			
VI		1.117 (0.017–9.937)	0.329 (0.001–7.589)	0.006		1.580 (0.115–6.031)	0.732 (0.001–9.937)	0.355
FI		33.598 (24.240–43.930)	30.788 (19.310–49.550)	0.057		31.811 (24.690–40.190)	32.232 (19.310–49.550)	0.538
VFI		0.353 (0.005–4.092)	0.105 (0–3.007)	0.008		0.556 (0.028–2.013)	0.232 (0–4.092)	0.396
*Automatic***								
Endometrium	68				66			
VI		0.766 (0–38.661)	0.031 (0–32.622)	0.003		1.449 (0.002–23.428)	0.228 (0–38.661)	0.207
FI		26.282 (0–47.105)	18.676 (0–44.151)	0.002		22.258 (16.187–41.937)	22.700 (0–47.105)	0.268
VFI		0.193 (0–16.709)	0.007 (0–14.403)	0.003		0.362 (0–9.825)	0.052 (0–16.709)	0.189
5-mm shell	90				88			
VI		1.484 (0–26.016)	0.390 (0–28.668)	0.020		1.731 (0.009–12.098)	0.765 (0–28.668)	0.212
FI		30.559 (0–43.667)	28.375 (0–54.078)	0.424		29.489 (18.598–36.986)	29.923 (0–54.078)	0.837
VFI		0.442 (0–10.924)	0.118 (0–11.365)	0.026		0.558 (0.002–4.475)	0.256 (0–11.365)	0.244
10-mm shell	94				90			
VI		1.378 (0–14.843)	0.633 (0.008–15.639)	0.043		1.475 (0.009–8.815)	0.961 (0–15.639)	0.285
FI		32.517 (0–44.901)	32.759 (20.371–49.143)	1.000		32.241 (19.935–37.195)	33.446 (0–49.143)	0.511
VFI		0.440 (0–6.336)	0.212 (0.002–6.094)	0.050		0.455 (0.002–3.045)	0.324 (0–6.336)	0.317

Data are presented as median (range). *Three cases of non-uterine histology excluded. †Three cases of non-uterine histology and four cases of unknown lymph node status excluded. ‡Number of patients with identified power Doppler signals. §Mann–Whitney *U*-test. ¶'Manual' option of VOCAL. **Automatic 'Sphere' option of VOCAL. FI, flow index; VFI, vascularization flow index; VI, vascularization index.

Table 4 Correlation of vascular indices with patient body mass index (BMI) and the distance between the probe and the center of the endometrium ($n = 97$)

	BMI		*Distance*	
Index	r	P*	r	P*
Manual†				
Endometrium				
VI	−0.073	0.503	−0.033	0.751
FI	−0.088	0.418	−0.049	0.634
VFI	−0.082	0.449	−0.037	0.716
5-mm shell				
VI	−0.193	0.073	−0.184	0.072
FI	−0.040	0.710	−0.097	0.344
VFI	−0.175	0.104	−0.177	0.082
10-mm shell				
VI	−0.311	0.003	−0.240	0.018
FI	−0.220	0.041	−0.126	0.220
VFI	−0.298	0.005	−0.230	0.024
Automatic‡				
Endometrium				
VI	−0.114	0.293	−0.229	0.024
FI	−0.104	0.336	−0.255	0.012
VFI	−0.118	0.275	−0.224	0.028
5-mm shell				
VI	−0.275	0.010	−0.272	0.007
FI	−0.321	0.002	−0.263	0.009
VFI	−0.278	0.009	−0.274	0.007
10-mm shell				
VI	−0.336	0.001	−0.210	0.039
FI	−0.309	0.004	−0.117	0.254
VFI	−0.341	0.001	−0.196	0.054

*Spearman's rho. †'Manual' option of VOCAL. ‡Automatic 'Sphere' option of VOCAL. FI, flow index; VFI, vascularization flow index; VI, vascularization index.

was detected. It is of particular interest that as many as 80% of the patients with no detectable endometrial vascularity did not have deep myometrial invasion.

In the multivariable analysis, the manually analyzed endometrial FI and endometrial volume were independently associated with deep invasion. Interestingly, in the study of Galván *et al.* the independent predictive factors were the endometrial volume and endometrial VI rather than FI[20]. The relatively high number of missing power Doppler signals in our study does not seem to explain this discrepancy, as the manually analyzed endometrial FI was left as the sole independent factor predicting deep myometrial invasion when the multivariable analysis excluded the patients with missing signals. Although the manually analyzed endometrial FI and endometrial volume were independent predictive factors in our multivariable analysis, their odds ratios were quite low.

We assessed the endometrial volume by outlining the endometrial margins, excluding any suspected areas of invasion. This method was chosen in order to evaluate the myometrial vasculature using the VOCAL shell application. However, excluding the areas of invasion may bias the calculation of endometrial volume and cause underestimation of the risk of deep myometrial invasion and metastatic disease.

The negative correlation of BMI with vascular indices seems to be an outcome of signal attenuation caused by the greater distance between the probe and the target tissue. Patients with a high BMI had larger uteri than patients with a low BMI, possibly due to a relative hyperestrogenism that is typical of obese women. Consequently, an increasing BMI was related to greater size of the uterus and increasing distance to the investigated area. To our knowledge, this is the first study to demonstrate the effect of signal attenuation of 3D-PDA in a clinical setting in cancer patients.

We did not exclude Grade 3 or other high-risk histology tumors from the analysis as we wanted to evaluate the use of 3D-PDA in detecting deep invasion regardless of the histology. Nevertheless, in clinical practice preoperative assessment of deep invasion is of little value if Grade 3 or high-risk histology is found prior to the operation. In spite of the result of the preoperative evaluation, a lymphadenectomy should be performed.

We chose to use a 15° rotational technique since it has been shown to be as accurate as the 9° technique, with the advantage of being less time-consuming[15,17]. Regarding the automatic 'Sphere' analysis, the vascularity inside the initial sphere that was set to match the margins of the endometrium presumably represents the vascularity in the endometrium. However, the performance of the surrounding shells in assessing the myometrial vascularity is probably partly compromised, since they unavoidably contain parts of the endometrium. This may cause the variability in the number of missing signals measured from the 5-mm and 10-mm shells when compared to manual analysis (Table 3). In spite of being aware of this inaccuracy, we wanted to evaluate the performance of the automatic analysis since it is easy to use and rapid, and thus potentially better suited for routine clinical practice than the manual method.

In the present study there were only eight patients with metastases. Although the mean vascular indices tended to be lower in the group with no metastases, the difference did not reach statistical significance, probably because of the low number of cases.

In conclusion, the results of this study indicate that endometrial volume and endometrial and myometrial vascular indices as measured by 3D-PDA correlate with the degree of myometrial invasion. 3D-PDA results are influenced by various factors. In order to make the results of different 3D-PDA studies comparable, there is a need for standardized machine settings. Moreover, since signal intensity is affected by the distance between the probe and target tissue, it should be taken into account when evaluating results.

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AOGS MAIN RESEARCH ARTICLE

The preoperative assessment of deep myometrial invasion by three-dimensional ultrasound versus MRI in endometrial carcinoma

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Key words
Endometrial carcinoma, magnetic resonance imaging, three-dimensional ultrasound, three-dimensional power Doppler angiography, staging

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Conflict of interest
The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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Abstract

Objective. To evaluate the usefulness of three-dimensional ultrasound (3D US), magnetic resonance imaging (MRI) and three-dimensional power Doppler angiography (3D-PDA) in the preoperative assessment of myometrial invasion in endometrial carcinoma. *Design*. A prospective observational study. *Setting*. University hospital. *Population*. Twenty consecutive patients diagnosed with endometrial carcinoma. *Methods*. Preoperative 3 T MRI and 3D US examinations were performed, and the depth of myometrial invasion was assessed. The vascularity indices, vascularization index, flow index and vascularization flow index, were calculated by 3D-PDA. *Main outcome measures*. The results were compared with the final histopathology report after a surgical staging. *Results*. In detecting deep myometrial invasion, the sensitivity of 3D US, MRI and their combination was 50, 91.7 and 100%, respectively. The specificity was 87.5, 50 and 50%, respectively. There were no significant differences in the 3D-PDA vascularity indices between the two groups. *Conclusions*. MRI appears to be more sensitive than 3D US in detecting deep invasion, while 3D US has a better specificity.

Abbreviations: BSO, bilateral salpingo-oophorectomy; 2D US, two-dimensional ultrasound; 3D-PDA, three-dimensional power Doppler angiography; 3D US, three-dimensional ultrasound; FI, flow index; LAE, pelvic lymphadenectomy; LH, laparoscopic hysterectomy; PALA, para-aortic lymphadenectomy; TSE, turbo spin echo; VFI, vascularization flow index; VI, vascularization index; VIBE, volumetric interpolated breath-hold examination.

Introduction

Endometrial carcinoma is the most common gynecological malignancy and it is the sixth most common malignancy among women worldwide. The incidence is highest in North America and Europe (1,2). Endometrial carcinoma is predominantly a disease of postmenopausal women, and postmenopausal vaginal bleeding is the most common diagnostic sign. As bleeding develops in the early stage of the

Key Message

Magnetic resonance imaging is more sensitive than three-dimensional ultrasound in detecting deep myometrial invasion in endometrial carcinoma, while three-dimensional ultrasound appears to have a better specificity.

disease, 75% of the carcinomas are diagnosed before an extrauterine spread occurs (3).

For early stage, low-grade tumors, hysterectomy with bilateral salpingo-oophorectomy is usually all that is needed to cure the patient. However, in patients with grade 3 tumors and/or deep (≥50%) myometrial invasion and/or cervical stromal spread, a pelvic and para-aortic lymphadenectomy is recommended, owing to a markedly increased risk of lymph node involvement (4). Grade can be quite reliably evaluated with preoperative endometrial sampling (5). However, preoperative estimation of myometrial invasion (and of cervical spread) has been challenging.

Transvaginal two-dimensional ultrasound (2D US) is commonly performed routinely for peri- and postmenopausal patients who present with vaginal bleeding. Transvaginal ultrasound has been used to detect deep invasion of an endometrial tumor with a sensitivity of 79–86.7% and a specificity of 75–100% (6–8). Applications of three-dimensional ultrasound (3D US) have also been studied for detection of deep invasion, with promising results. The possibility of reviewing the stored 3D volumes in any desired plane has improved the accuracy of the evaluation of tumor invasion (9). Assessment of tumor vasculature by a combination of 3D volume US and power Doppler, 3D-power Doppler angiography (3D-PDA), has been studied in gynecological oncology but has not yet proven clinical effectiveness (10–13).

Cross-sectional imaging modalities, computerized tomography and magnetic resonance imaging (MRI) have also been used in preoperative staging of endometrial carcinoma. MRI provides excellent soft tissue contrast resolution, with multiplanar capabilities, when evaluating female pelvic tumors, and it is the most widely used imaging modality for preoperative staging of endometrial cancer. The primary purpose of preoperative MRI is to assess myometrial and cervical stromal invasion, as well as lymph node metastases (14).

The sensitivity and specificity of MRI in detecting deep myometrial invasion has ranged from 50 to 93% and from 82 to 100%, respectively (15–21), with a negative predictive value of 66–92% (22–24). The sensitivity and specificity of MRI in the detection of cervical invasion has been in the order of 19–87.5% and 47–100%, respectively (15,18,22–25). In assessing extrauterine spread, MRI finds an invasion of the bladder and/or rectum with a sensitivity of 100% and a specificity of 99% (22).

Intraoperative gross evaluation is an alternative way to assess the degree of myometrial invasion and, compared with preoperative imaging methods, it is very similar in terms of sensitivity and specificity (26).

The aim of this study was to compare the performance of 3D US, 3D-PDA and MRI in detection of deep myometrial invasion of endometrial carcinoma. The depth of invasion was confirmed histologically from the hysterectomy specimens, after a surgical staging involving a pelvic or pelvic and para-aortic lymphadenectomy, as well as hysterectomy and bilateral salpingo-oophorectomy.

Material and methods

Twenty patients with endometrial carcinoma scheduled for operation at Tampere University Hospital between September 2008 and July 2009 were enrolled. All patients provided written informed consent, and the study protocol was accepted by the local Ethics Committee. All patients were scheduled for a hysterectomy, bilateral salpingo-oophorectomy, pelvic lymphadenectomy (also para-aortal if indicated) and peritoneal fluid sampling. The patients were originally staged according to the FIGO 1988 recommendations, but were restaged for the purpose of this study according to the FIGO 2009 recommendations (27,28). The MRI examination was performed 1–17 days and the ultrasound examination 24 h prior to the operation.

Ultrasound examination

All of the ultrasound examinations were performed by using a Voluson 730 Expert (GE Medical Systems, Zipf, Austria) with a multifrequency endovaginal probe (5–9 MHz) by one of the authors (S.K.S.) with two years of experience in 3D ultrasound. The transvaginal ultrasound examination was performed with an empty bladder in the lithotomy position. After routine B-mode evaluation, 3D-power Doppler was used to assess myometrial vascularization. Power Doppler settings used were as follows: frequency 5 MHz, power Doppler gain –0.6, dynamic range 20–40 dB, persistence 2, color map 5, WMF filter low 1, PRF 0.6 kHz, rise 5 and fall 5. Acquisition sweep angle was set to 75–85 degrees, depending on the size of the uterus.

Once a 3D volume containing the uterus was obtained, it was stored on a hard disk (Sonoview; GE Medical Systems). The volume was briefly analysed visually, and if it did not fulfill the requirements (no artifacts caused by movement, the whole uterus inside the volume box), a new volume was obtained. The stored volumes were later analysed by the same examiner (S.K.S.) using the 4DView Software (version 9.1; GE Medical Systems). Using the virtual organ computer aided analysis (VOCAL) utility, the uterine volume was estimated by outlining the serous margins of the uterus inside the volume box in the plane A. The volume was measured from the cervicoisthmic region towards the fundus using the 'Manual' option. The measurements were done with 15 degree rotations. Shells 5 and 10 mm thick were then created inside the selected volume using the automatic 'Edit Region of Interest' facility. The vascularity indices, vascularization index (VI), flow index (FI) and vascularization flow index (VFI), were calculated from the selected shells using the histogram facility.

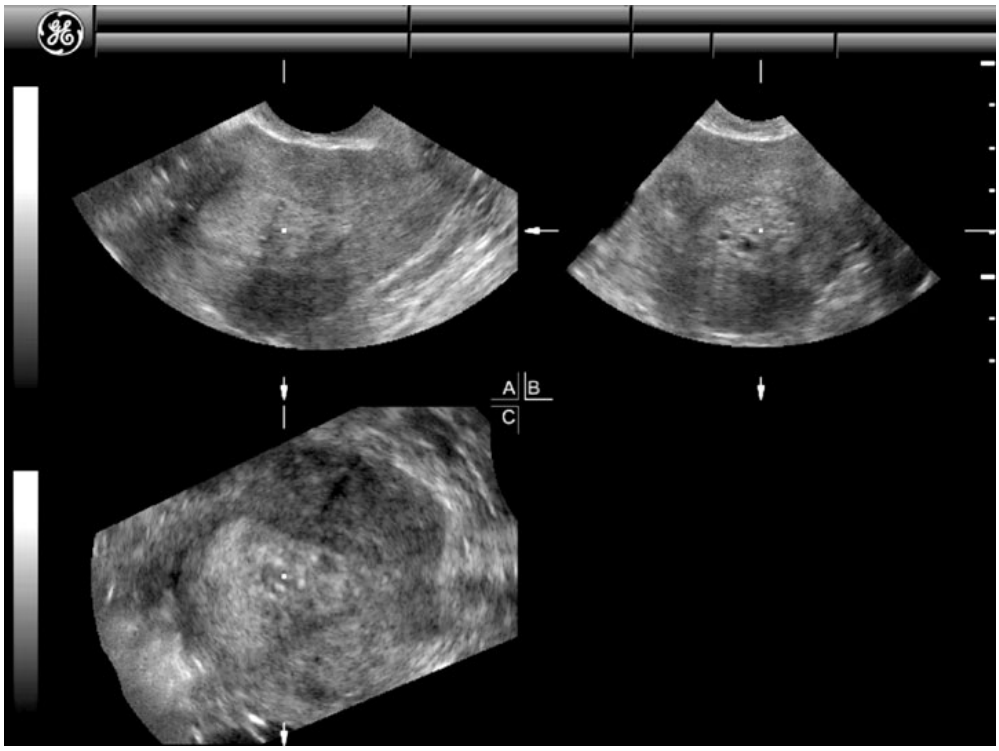

Figure 1. A three-dimensional ultrasound image of the uterus in Patient no. 18, with no deep invasion, demonstrating sagittal (A), transverse (B) and rendered coronal planes (C) of the 'Sectional planes' utility. When moving through planes A and B, the margins of the endometrial tumor can be assessed thoroughly.

After 3D-power Doppler analysis, Doppler signal color was switched off, and the acquired volume was assessed using the 'Sectional planes' utility. The volume was rotated until sagittal, transverse and coronal planes of the uterus were placed on planes A, B and C, respectively. The assessment of the myometrium was then performed by going through planes A and B. A maximal depth of myometrial invasion was estimated from planes A or B based on the subjective impression of the examiner (Figure 1).

Magnetic resonance imaging

After a six hour fast, the patient emptied the bladder immediately before imaging. Twenty milligrams of butylscopolamine (Boehringer Ingelheim, San Cugat del Vallés, Spain) was administered intravenously (unless contraindicated) to reduce artifacts caused by peristaltic movements. The vagina was filled with Thicken Up Gel® (Milupa GmbH, Fulda, Germany) to improve the evaluation of possible cervical invasion. Magnetic resonance imaging was performed with a Magnetom Trio a Tim System 3 T scanner (Siemens, Erlangen, Germany), using a six-channel Body Matrix coil. In all cases, a coronal turbo spin echo (TSE) T1-weighted image (TR/TE 700/22, 5 mm sections and 40 cm field of view), an axial (oblique to the uterus) TSE T2-weighted image (TR/TE 4180/69, 5 mm sections and 38 cm field of view), a parasagittal TSE T2-weighted image (TR/TE 5000/86, 4 mm sections and 24 cm field of view, sample matrix size 320 × 320) and a paracoronal (axial to the uterus) TSE T2-weighted image (TR/TE 6000/114, 3 mm sections and 25 cm field of view) were acquired. The dynamic MRI scans were performed with a rapid bolus injection of 15 ml gadolinium-tetraazacyclododecanetetraacetic acid (Gd-DOTA; Guerbet, Roissy, France) 279.3 mg/mL using the 3D volumetric interpolated breath-hold examination (VIBE; TR/TE 3.48/1.28, 28.5 cm × 38 cm field of view, flip angle 10, voxel size 1.8 mm × 1.5 mm × 2 mm, matrix size 157 × 256). These images were obtained in the paracoronal plane before and at 30 and 60 safter administration of the contrast medium. A parasagittal fat-suppressed TSE T1-weighted image (TR/TE

600/11, 4 mm sections and 24 cm field of view) was performed 120 safter contrast injection. The MRI findings were analysed as consensus reading by two radiologists experienced in oncological MRI (L.K. and R.J.). Initially, all images from each imaging session were analysed, including: (i) tumor signal intensity in each image; (ii) continuity of junctional zone on T2 images; (iii) depth of tumor invasion in myometrium (from parasagittal T2-weighted image, paracoronal T2-weighted image and from postcontrast images); (iv) size of tumor; (v) cervical invasion; and (iv) pelvic lymph nodes (from large field of view sequences). After all patients were examined, a second blinded reading (the same interpreters as in the first reading) was made by measuring the depth of the estimated tumor invasion in 3D VIBE images. The volume analysis was not used.

The ultrasound examination and the 3D volume analysis were performed blinded to the results of the MRI and the pathology report. The histological samples were reviewed by a pathologist specialized in gynecological pathology (M.L.).

Statistical analysis

The statistical analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to evaluate normal distribution of continuous variables. The comparison of the groups was made using Mann–Whitney *U*-test when appropriate.

Table 1. Patient demographics, surgical staging procedures and final diagnoses.

Patient no.	Age (years)	Operation	Stage*	Grade†
1	70	LH + BSO + LAE	IA	1
2	66	LH + BSO + LAE	IB	1
3	65	LH + BSO + LAE	IA	1
4	69	LH + BSO + LAE + PALA	IB	3
5	67	LH + BSO + LAE	IA	2
6	77	LH + BSO + LAE	IB	2
7	81	LH + BSO + LAE	IA	1
8	59	LH + BSO + LAE	IB	2
9	65	LH + BSO + LAE	IB	3
10	70	LH + BSO + LAE + PALA	IIIC1	1
11	67	LH + BSO + LAE	IB	1
12	67	LH + BSO + LAE + PALA	IA	3
13	70	LH + BSO + LAE	IB	3
14	78	LH + BSO + LAE	IB	1
15	67	LH + BSO + LAE + PALA	IA	2
16	62	LH + BSO + LAE + PALA	IA	3
17	70	LH + BSO + LAE	IB	1
18	74	LH + BSO + LAE	IA	1
19	69	LH + BSO + LAE + PALA	IB	3
20	61	LH + BSO + LAE	IIIA	1

Abbreviations: BSO, bilateral salpingo-oophorectomy; LAE, pelvic lymphadenectomy; LH, laparoscopic hysterectomy; and PALA, para-aortic lymphadenectomy.

* FIGO 2009 stage.

† Grade 3 patients include one serous adenocarcinoma.

Results

The mean (±SD) and median ages of the patients were 68.7 ± 5.56 and 68.0 years (range 59–81 years), respectively. The patient demographics, the surgical staging procedures and the final diagnoses based on the histopathology reports are presented in Table 1.

The performance of 3D US and MRI, and their combination is shown in Tables 2 and 3, respectively. Although MRI was more sensitive, 3D US was more specific. Of note is the fact that in all eight patients with either no myometrial invasion or with invasion into the inner half of the myometrium only, the disease was limited to the uterine corpus (FIGO 2009 stage IA disease). Of the remaining 12 patients, 10 had stage IB disease (FIGO 2009 classification), with one case (detected by neither MRI nor 3D US) of endocervical glandular involvement, which, according to the FIGO 2009 classification, does not change the stage from IB.

In three women, the evaluation by 3D US was impaired owing to uterine fibroids. As no signs of deep invasion were detected, the result was considered to be negative. Two of these patients had deep myometrial invasion. The MRI assessment was superior in these cases by detecting deep invasion correctly (Figure 2).

The mean vascularity indices measured by 3D-PDA are presented in Table 4. Although all indices were numerically lower in the group with no or low myometrial invasion, the differences were not statistically significant. A statistically significant correlation was not found when the vascularity indices were correlated with the grade of the tumor.

The depth of myometrial invasion in 3D MRI VIBE images was assessed correctly in 13 (65%) of 20 patients, overestimated in three and underestimated in four patients, respectively.

Discussion

In this study, MRI had a high, 91.7% sensitivity, but a poor, 50% specificity in detecting the depth of myometrial invasion. The opposite held true for 3D US, with a sensitivity of 50% and a specificity of 87.5%. When both methods were combined, all cases of deep myometrial invasion were found preoperatively, with a negative predictive value of 100%.

Alcázar et al. evaluated the performance of 3D virtual navigation in detection of a deep myometrial invasion. The measurement of the myometrial tumor-free distance to the serosa by 3D ultrasound had a negative predictive value of 100%, which far exceeds the performance of 2D US. The performance of detection by the examiner's subjective impression was somewhat poorer but still very good, at 96.6% (9). Unfortunately, the report of Alcázar et al. was not available until the

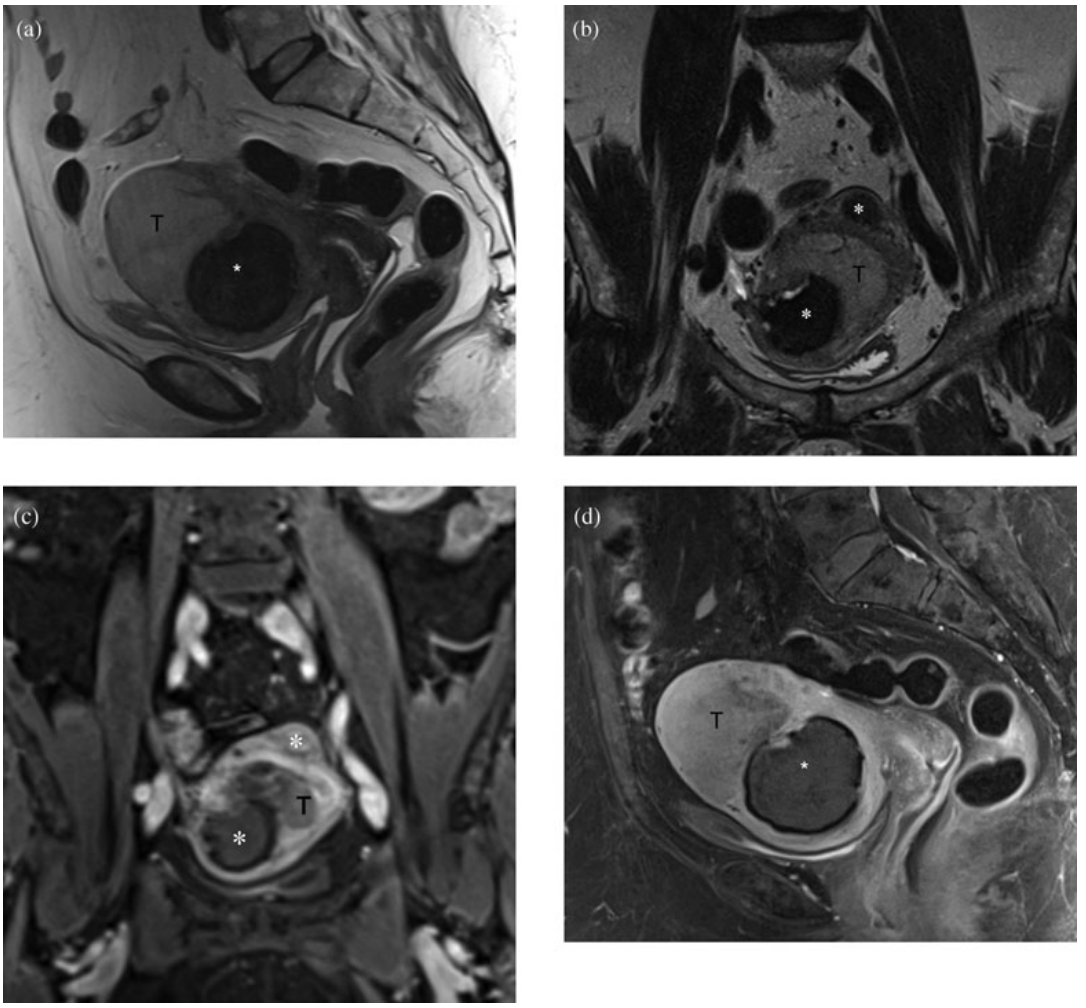

Figure 2. (a) Sagittal turbo spin echo (TSE) T2-weighted image of the uterus in Patient no. 19. The evaluation of tumor invasion by three-dimensional ultrasound was impaired because of a large leiomyoma (*). Magnetic resonance imaging demonstrates endometrial tumor (T) invading more than 50% of the myometrial thickness. (b) Paracoronal T2-weighted image (perpendicular to the cavity of uterus). (c) Paracoronal contrast-enhanced three-dimensional volumetric interpolated breath-hold examination image acquired 60 safter contrast injection. (d) Sagittal contrast-enhanced fat-suppressed TSE T1-weighted image acquired two minutes after contrast injection demonstrates a higher contrast between the tumor and myometrium.

recruitment to the present study was already ongoing; therefore, we had to rely on the subjective impression. Moreover, we had chosen to obtain the 3D volumes with power Doppler, which makes the volume acquisition time longer and can therefore cause artifacts. The methods used by Alcázar et al. were, however, inferior to the ones used by us in terms of specificity, which in their study ranged from 61 to 82.3%, in comparison to our 87.5%.

It has previously been reported that endometrial vascularity indices measured by 3D-PDA are higher in patients with endometrial carcinoma compared with benign conditions (29). Our results suggest that vascularity indices in the myometrium may, on the contrary, be influenced by a deep invasion of the tumor. The vascularity indices were numerically, albeit not significantly, higher when a deep invasion was present. It is possible that the lack of statistical

Table 2. The presence of deep myometrial invasion in 20 patients with endometrial carcinoma according to the final histopathology report, and its comparison with the findings of 3D US, MRI, or both.

Modality	Histology		
	+	–	*n*
3D US			
+	6	1	7
–	6	7	13
n	12	8	20
MRI			
+	11	4	15
–	1	4	5
n	12	8	20
3D US + MRI*			
+	12	4	16
–	0	4	4
n	12	8	20

Abbreviations: 3D US, three-dimensional ultrasound; and MRI, magnetic resonance imaging.
* Considered positive if either one or both show deep invasion.

Table 3. Comparison of the performance of MRI, 3D US, or their combination in predicting the depth of myometrial invasion correctly.

Modality	Sensitivity (%)	Specificity (%)	Negative predictive value (%)	Positive predictive value (%)
3D US	50.0	87.5	53.8	85.7
MRI	91.7	50.0	80.0	73.3
3D US + MRI	100	50.0	100	75.0

Abbreviations: 3D US, three-dimensional ultrasound; and MRI, magnetic resonance imaging.

significance was due to the rather small sample size only. However, margins of the uterus can be difficult to visualize in the 2D US examination in endometrial carcinoma patients, and this problem is present in 3D examinations as well, particularly when there are multiple leiomyomas. As evaluation of vascularity indices with this method requires that the myometrium first has to be outlined from the 3D volume, there is the possibility of an error. There are also other technical problems to be solved before implementing 3D-PDA indices to the evaluation of myometrial invasion. Ultrasound machine settings have an effect on vascularity indices, and probably the greatest problem is the attenuation of the ultrasound signal. So far, there are no methods available to compensate for the effect of the distance between the probe and target tissue. As there are no standardized settings for 3D-PDA, no cut-off values for a positive or negative test result for a deep invasion can be set.

Previous studies have suggested that MRI has a high diagnostic accuracy in evaluation of the depth of myometrial infiltration (25). However, contradictory studies have also been published (22). Patient-related causes of misdiagnoses include the following: tumor isointensity with the myometrium; polypoid tumor; myometrial thinning; irregular myometrium; and presence of adenomyosis or leiomyomas (15). Furthermore, there is a possibility of various MRI artifacts (abdominal wall movement, peristalsis, magnetic susceptibility artifact, chemical shift and dielectric effect) affecting image quality (16).

In the present study, MRI correctly estimated 11 (91.7%) of 12 patients with deep myometrial invasion, and underestimated only one of them. In this patient, there were multiple leiomyomas causing distraction of the myometrium. There were four patients in whom MRI overestimated the myometrial invasion. Ryoo et al. pointed out that large tumors tend to diminish the myometrial thickness, leading to more frequent false-positive or false-negative results (19). This phenomenon can explain two of our four cases of overestimation. In one false-positive case, the endometrial tumor was accompanied by adenomyosis.

Sensitivity of measuring the myometrial invasion only in 3D VIBE images was lower, at 75%. A voxel size of 2 mm and a matrix of 157 × 256 in the 3D VIBE images were used in the

Table 4. The median vascularity indices measured by three-dimensional power Doppler angiography (3D-PDA).

Index	Deep myometrial invasion		
	Yes	No	*p*-Value*
5 mm shell VI	1.178(0.056–8.451)	0.654(0.146–3.791)	0.251
5 mm shell FI	34.401(32.115–43.081)	33.855(24.866–42.226)	0.405
5 mm shell VFI	0.428(0.018–3.641)	0.227(0.037–1.284)	0.285
10 mm shell VI	1.217(0.050–10.650)	0.594(0.130–2.777)	0.285
10 mm shell FI	34.731(30.479–41.324)	33.120(24.566–41.769)	0.501
10 mm shell VFI	0.374(0.015–4.401)	0.208(0.032–0.920)	0.285

Note: Data are presented as medians (range). The median distance from the surface of the probe to the center of the target tissue was 2.34 cm (range 1.58–3.53 cm). Abbreviations: FI, flow index; VFI, vascularization flow index; and VI, vascularization index.
* Mann–Whitney *U*-test.

present study. Apparently, they were not accurate enough to allow for sufficient spatial resolution to evaluate the relatively small tumor changes in the myometrium. Rapid dynamic imaging is required in order to increase the time resolution, at the cost of a lower spatial resolution. A higher contrast between the tumor and the myometrium was noticed subjectively in contrast-enhanced spin echo T1-weighted images performed after 120 s after injection of contrast medium. This may be partly an effect of better spatial resolution in spin echo T1-weighted images. However, according to Manfredi et al., the optimal contrast-to-noise ratio between the endometrial tumor and normal myometrium is at 150 safter administration of gadolinium (30). In the European Society of Urologenital Imaging Guidelines for staging endometrial cancer with MRI, the authors have stated that postcontrast images acquired at 120 ± 30 safter CE injection are suggested to be optimal for diagnosis of myometrial invasion (31). We started with the examinations in 2008, and this guideline was not yet available. The newer guideline might have helped us to obtain better results.

Conclusion

The results of this preliminary study imply that MRI is more sensitive than 3D US in detecting myometrial invasion of endometrial carcinoma. However, leiomyomas seem to be an obstacle for both imaging methods. It seems that 3D VIBE is not sensitive enough to be used instead of a complete analysis of the magnetic resonance images. These preliminary results imply that 3D US can be used to screen for a deep invasion, with a high positive predictive value. However, especially in the presence of simultaneous leiomyomas, a negative result should be interpreted with caution. In these cases, a supplementary MRI seems to increase the negative predictive value (up to 100%). This kind of sequential imaging may potentially decrease the need to perform a lymphadenectomy, provided that these results are confirmed in future, larger studies.

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Predictive value of serum human epididymis protein 4 and cancer antigen 125 concentrations in endometrial carcinoma

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Abstract

Objective: The purpose of this study was to evaluate the performance of preoperative serum levels of human epididymis protein 4 (HE4) and cancer antigen 125 (CA125) in prediction of the presence of metastases in endometrial carcinoma.

Study design: Preoperative sera were collected from 98 women diagnosed with endometrial carcinoma. The concentrations of HE4 and CA125 were assessed by enzyme-linked immunosorbent assay and correlated to the results of the final histopathological report.

Results: Fourteen patients had metastases (≥Stage IIIA, FIGO 2009 classification). The serum concentrations of HE4 and CA125 were higher in the group with metastases than in the group without metastases (median [interquartile range], 148.6 pM [71.6–219.1 pM] vs. 77.2 pM [52.9–99.3 pM]; *P*=.001 and 20.0 U/mL [10.1–70.8 U/mL] vs. 4.3 U/mL [2.9–10.4 U/mL]; *P*<.001, respectively). By a multivariate analysis, the combination of HE4 and CA125, a risk score algorithm, was the only predictive factor for the presence of metastases (odds ratio, 21.562; 95% confidence interval, 5.472–84.963; *P*<.001), and grade was the predictor for a deep (≥50%) myometrial invasion by the tumor (odds ratio, 2.005; 95% confidence interval, 1.123–3.581; *P*=.019). The sensitivity, specificity, positive predictive value and negative predictive value for the combination of the markers to predict the presence of metastases were 71.4, 89.5, 55.6 and 94.4, respectively.

Conclusion: A combination of preoperative HE4 and CA125 seems to be a better predictor of metastatic disease than either one alone in endometrial carcinoma.

Key words: Endometrial carcinoma, HE4, CA125, staging, ELISA

Introduction

Endometrial carcinoma is the most common gynecological malignant tumor in the developed countries. Endometrial carcinoma has generally a good prognosis, mainly because only a minority (25%) of the patients has a metastatic disease at presentation. The treatment of endometrial carcinoma is surgical, including a hysterectomy, bilateral salpingo-oophorectomy, peritoneal fluid sampling, and a pelvic and periaortic lymphadenectomy.[1] However, the need for a routine lymphadenectomy has recently been debated in the case of the low-risk or Stage IA Grade 1–2 disease.[2, 3]

Serum markers are available for the diagnosis and follow-up of several cancers. CA125 that is commonly used in ovarian cancer, has been investigated also for endometrial carcinoma.[4–6] A cut-off limit discriminating normal and pathological serum levels like the one for ovarian cancer or 35 U/mL, has not yet been defined. However, an elevated CA125 level may be associated with a metastasized disease and serial CA125 measurements can also be used during the treatment and follow-up of patients.

Human epididymis protein 4 (HE4) was originally isolated from the human epididymis but is also expressed in other tissues of the body.[7, 8] The HE4 protein contains two whey acidic protein (WAP) domains and a core of four disulphides. Since its introduction, the biomarker capability of HE4 has been studied in various malignant tumors, including gastric, breast and lung cancer.[9–11] In endometrial carcinoma, the serum concentration of HE4 has been shown to correlate with the depth of myometrial invasion and the stage of the disease.[12–14] Currently, the analysis of the concentration of HE4 in serum is used in parallel with CA125 to detect ovarian cancer, especially in premenopausal women.[15] HE4 has also been shown to help in discriminating benign endometriotic cysts from malignant ovarian tumors.[16]

The aim of this study was to investigate the performance of the preoperative serum HE4 and CA125 levels in predicting an advanced stage and/or a deep (≥50%) myometrial invasion in endometrial carcinoma.

Materials and methods

Ninety-eight consecutive women with a newly diagnosed endometrial carcinoma and treated at Tampere University Hospital between Sep 2007 and Oct 2009, were enrolled in this prospective observational study. All patients gave written informed consent; the study was approved by the Ethics Committee of the Pirkanmaa Hospital District. All patients were scheduled for a hysterectomy, bilateral salpingo-oophorectomy, pelvic lymphadenectomy, and peritoneal fluid sampling. A para-aortic lymphadenectomy and an infracolic omentectomy were performed when indicated. Preoperative serum samples were collected at the preoperative outpatient office visit or on the day preceding the operation, and stored at -70 °C until analyzed.

The HE4 and CA125 concentrations in serum samples were measured by sandwich enzyme-linked immunosorbent assay (ELISA) kits (Fujirebio Diagnostics inc., Malvern, PA; and Abnova GmbH, Heidelberg, Germany, respectively). All measurements were performed at room temperature according to the manufacturers' instructions blinded to the results of the histopathological report. The plates were read two and five minutes after the administration of the stop solution at the wavelength of 405 nm for HE4 and 450 nm for CA125, respectively. The minimum detection limits for HE4 and CA125 were 15 pM and 5 U/mL, respectively. The coefficient of variance (CV%) was 6.9% for HE4, and 13.6% for CA125, respectively.

The results of the ELISA analysis were correlated with the final histopathological report. The patients were originally staged according to the

FIGO 1988 guidelines, but for the purpose of this study they were re-staged according to the new 2009 guidelines.[17]

The statistical analysis was performed using SPSS software (version 18.0; SPSS, Chicago, IL) and MedCalc software (version 12.0; MedCalc Software, Mariakerke, Belgium) softwares. The distribution of the continuous variables was assessed using the Kolmogorov-Smirnov test. The comparison of the groups was performed using the Mann-Whitney U-test and the Kruskal-Wallis test when appropriate. The Spearman's rank correlation test was used to evaluate correlations between the continuous variables. To compare the predictive performance of the markers, a receiver operating characteristics (ROC) analysis was accomplished. The variables that were found to be statistically significant in the univariate analysis were compared in a multivariate regression analysis by defining the dependent variables as metastasis or non-metastasis and the presence or absence of a deep (≥50%) myometrial invasion. A probability value of <.05 was considered as statistically significant. All tests were two-sided.

Results

The mean age of the patients was 66.8 ± 8.8 years (range, 33–87 years). The demographics of the patients and the histopathological characteristics of the tumors are presented in Table 1. Three patients had a non-uterine cancer in the final histopathological report, although the preoperative diagnosis based on the endometrial biopsy or curettage specimen had been endometrial carcinoma. Of these cases, one was a cervical cancer, one was a Fallopian tube cancer and one was an endometrioid ovarian cancer. These three patients were excluded from the study. In five operations, the surgeon refrained from performing a lymphadenectomy because of the obesity or co-morbidity of the patient. One of these patients had metastases in the ovaries. The other four women were excluded

from the statistical analysis when the variables were correlated with respect to the presence of metastases.

The median concentrations of HE4 and CA125 in serum are presented in Tables 2 and 3. Both markers exhibited a correlation with metastases and a deep myometrial invasion. An association with the patient´s age and body mass index (BMI) was observed for the concentration of HE4, whereas no similar association was seen for CA125. A positive correlation was found between the levels of HE4 and CA125 (Table 4). The HE4 levels furthermore correlated with the histological grade (median [interquartile range] 69.8 [51.3–101.9] vs. 77.2 [52.7–95.3] vs. 99.2 [64.7–172.3]; Grades 1, 2 and 3; respectively; *P*=.012, Kruskal-Wallis test). However, when the analysis was repeated excluding the metastatic cases, no statistically significant correlation was found.

With cut-off limits of 70 pM and 35 U/mL for HE4 and CA125, respectively, the sensitivity, specificity, positive predictive value and negative predictive value for the presence of metastases were calculated for both markers. The performance of the combination of the markers was evaluated by calculating a risk score for the presence of metastases using the algorithm for postmenopausal women that had been described by Moore et al.[15] A risk score of >27.7 was considered positive. As two of the three premenopausal patients were analogous to the postmenopausal group by their age (49 and 51 years, respectively), they were included in the risk score analysis. The youngest of the study participants (age 33 years) was excluded (Table 5). Nineteen women had a risk score of >27.7. All of them had a deep myometrial invasion and ten had also a metastatic disease. A ROC analysis for the risk score algorithm produced an area under the curve of 0.824 (95% confidence interval [CI], 0.689–0.959) for the presence of metastases. When this was compared to AUCs of HE4 and CA125 as single markers (0.763 [95% CI, 0.610–0.917] and 0.802 [95% CI, 0.672–0.932]), no statistical difference was found (risk

score vs. HE4; risk score vs. CA125; HE4 vs. CA125; *P*=.170; *P*=.424; *P*=.545, respectively).

The multivariate analysis left the risk score algorithm as the sole independent predictor for the presence of metastases (odds ratio [OR], 21.562; 95% CI, 5.472–84.963; *P*<.001, forward stepwise logistic regression analysis). Grade was independently associated with the presence of a deep invasion (OR, 2.005; 95% CI, 1.123–3.581; *P*=.019, forward stepwise logistic regression analysis).

Comment

The role of the preoperative risk assessment is fundamental because there are still many controversies regarding the role of lymphadenectomy. A lymphadenectomy increases both the time of the operation and blood loss, and it may cause a lower extremity lymphedema with an adverse effect on the patient's quality of life. Some studies have indicated that a surgical removal of lymph nodes is beneficial and should be performed, whereas other groups have reported opposite results.[18–22] The at present available preoperative risk factors assessed by imaging or histologic evaluation do not sufficiently aid the operating surgeon in the process of decision making regarding the lymphadenectomy. Therefore there is a need for adjuvant methods to assess an individual patient's risk profile.

In the present study, both HE4 and CA125 correlated with the presence of metastases and a deep myometrial invasion. The statistical analysis did not show a clear superiority for either marker. Although the ROC analysis favored CA125, the difference between the markers did not reach a statistical significance. However, according to our results, the use of HE4 in a clinical setting could be more feasible than CA125, as when the levels of the markers are investigated, only seven patients had a CA125 level above the threshold or 35 U/mL. Four of them had metastases. The median level of CA125 remained under the threshold in all grades regardless

of metastases. Regarding the ELISA analysis, the relatively high coefficient of variance of CA125 is presumably a result of the preponderance of low concentrations of the marker.

The distinction between normal and pathologic values was neither clear for HE4. The median level was <70 pM, which is a cut-off used in ovarian cancer, only in the case of the well-differentiated nonmetastatic tumors. Nevertheless, in all grades, the median levels of HE4 in the metastatic group were clearly >70 pM.

Our results imply that the combination of the HE4 and CA125 analysis with a risk score algorithm improves the diagnostic value of the markers, compared with the separate analyses. The result of the multivariate analysis is supported by the outcome of the ROC analysis in which the risk score algorithm produced a greater, although not statistically significant, area under the curve for the prediction of metastases when compared with HE4 and CA125 alone. For well and moderately differentiated endometrial carcinomas a normal preoperative risk score could advocate a mere hysterectomy and bilateral salpingo-oophorectomy without a lymphadenectomy, when preoperative imaging presents no conflicting results. In any respect, for grade 1 tumors the probability of a metastatic disease at presentation is relatively low (range, 0–7%).[23, 24] For moderately differentiated tumors the probability is to a degree higher (range, 0–17%).[24] Hence, the relevance of a preoperative risk scoring is emphasized particularly in the case of grade 2 tumors (negative predictive value, 94.7; Table 5).

The diagnostic and predictive value of the measurement of serum HE4 in endometrial carcinoma has been assessed in several recent studies.[12–14, 25, 26] Bignotti et al. found that serum HE4 concentration correlated with the FIGO stage, presence of a deep myometrial invasion, and the histological grade. In their study, the association between the HE4 levels and grade was observed only when Grade 1 tumors were compared with grade 2 or grade 2+3 tumors.[25] In the studies by Kalogera et al., Mutz-Dehbalaie et al. and Angioli et al., no such correlation

between the HE4 levels and grade was found.[14, 26, 27] Metastases, which are more likely to occur with an advancing grade, may bias the results when the association of HE4 and grade is evaluated. In our study, the correlation was no longer found when the metastatic cases were excluded.

In the present study, the median levels of HE4 in the metastatic and non-metastatic groups were comparable to the results of Moore et al. and Bignotti et al.[13, 25] However, we found a higher median concentration for the metastatic group than the previous two studies did. This difference may be a result of demographic and histological differences in the study populations because all measurements in the studies were carried out using a commercial ELISA kit by the same manufacturer.

In a recent study, Moore et al. reported a marked increase of HE4 levels with increasing age.[28] In their study, women who were >80 years old exhibited almost two-fold higher serum concentrations of HE4 than women in the age group of 60–69 years. In our study, the HE4 levels correlated with the age of the patients in a similar manner. Although age-specific cut-off levels are yet to be established, one has to take into account the effect of the patient's age when interpreting the results of a HE4 measurement.

We observed a positive correlation between BMI and the HE4 levels of the patients. Of the previous studies of HE4 and endometrial carcinoma, only Bignotti et al. reported a comparison of BMI and the HE4 concentration.[25] Conversely, in their study, a correlation was not seen between the two variables. In a study of >1500 healthy subjects, Bolstad et al. found that subjects with a lower BMI exhibited higher HE4 concentrations; they also noticed an association with smoking and high HE4 levels.[29] Compared with our study, the population in their study was notably different: median age, 48 years; included both male and female subjects. Regarding smoking habits, smokers tend to have a lower BMI than nonsmokers, which may bias results.[30] We did not record the smoking habits of

the patients in our study; thus a comparison cannot be made. However, the following confounding factors must be recognized. The effect of BMI on HE4 in a cohort of patients with endometrial carcinoma may be biased because obesity is a risk factor of type I endometrial carcinoma that represents most of the cases. Also, the correlation of the age and BMI of the patients may have an influence on the results.

In conclusion, our results suggest that a risk score calculation by a combination of preoperative measurements of serum HE4 and CA125 could be used as an adjunct to present methods when the risk for metastases in endometrial carcinoma is evaluated.

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Table 1. Patient demographics and histological characteristics (n=95 women).

Age (y)	66.8 ± 8.8 (33-87)
Weight (kg)	80.6 ± 16.3 (52-130)
Body mass index (kg/m²)	30.3 ± 6.0 (20.3-46.1)
Premenopausal	3
Postmenopausal	92
Histology (n)	
Endometrioid	86
Serous	4
Clear cell	1
Mixed	2
Carcinosarcoma	2
Grade (n)	
1	40
2	24
3	31
Stage (n)[a]	
I	
A	47
B	28
II	6
III	
A	6[c]
B	0
C	6
IV	
A	0
B	2
Myometrial invasion (n)	
<50%	50
≥50%	45
Personal history of malignancy[b] (n)	6

Data are presented as mean ± SD (range) unless otherwise indicated; [a]FIGO 2009 classification; [b]Breast cancer; [c]Metastases in the ovaries.

Table 2. Serum concentrations of HE4 and CA125 according to the presence of metastases.

Grade	**n**[b]		**HE4 (pM)**			**CA125 (U/mL)**		
	Metastases		Metastases		*P*[a]	Metastases		*P*[a]
	No	Yes	No	Yes		No	Yes	
1	34	4	64.1 (48.9-99.0)	128.8 (73.3-415.7)	0.052	4.3 (2.2-7.0)	25.3 (8.6-115.4)	0.023
2	21	3	77.2 (52.7-92.6)	148.3 (50.9-)	0.310	4.2 (3.2-8.3)	28.9 (14.6-)	0.023
3	22	7	91.9 (63.4-130.3)	174.9 (74.4-250.1)	0.067	4.8 (2.7-12.4)	14.4 (4.5-57.6)	0.083
All	77	14	77.2 (52.9-99.3)	148.6 (71.6-219.1)	0.001	4.3 (2.9-10.4)	20.0 (10.1-70.8)	<0.001

Data are presented as median (interquartile range) unless otherwise indicated; [a]Mann Whitney U test; [b]four patients with unknown lymph node status excluded; HE4, human epididymis protein 4; CA125, cancer antigen 125.

Table 3. Serum concentrations of HE4 and CA125 according to the presence of a deep myometrial invasion.

Grade	n		**HE4 (pM)**			**CA125 (U/mL)**		
	Deep invasion		Deep invasion		*P*[a]	Deep invasion		*P*[a]
	No	Yes	No	Yes		No	Yes	
1	26	14	63.5 (45.7-93.7)	83.4 (61.5-145.6)	0.039	3.4 (1.8-6.1)	10.8 (4.7-23.8)	0.001
2	15	9	75.2 (52.7-83.4)	89.6 (47.3-169.9)	0.290	4.3 (3.2-10.2)	4.2 (3.2-40.5)	0.446
3	9	22	70.3 (55.9-91.9)	122.6 (81.6-207.7)	0.009	3.2 (2.3-4.0)	11.2 (4.7-20.5)	0.002
All	50	45	68.3 (50.5-90.1)	111.7 (63.3-164.7)	<0.001	3.6 (2.2-5.4)	10.9 (4.1-25.3)	<0.001

Data are presented as median (interquartile range) unless otherwise indicated; [a]Mann Whitney U test; HE4, human epididymis protein 4; CA125, cancer antigen 125.

Table 4. Correlation of the markers with the age and BMI of the patients.

	HE4		CA125	
	r	P^a	r	P^a
Age	0.406	<0.001	0.078	0.452
BMI	0.406	<0.001	-0.058	0.614
HE4			0.404	<0.001
CA125	0.404	<0.001		

[a]Spearman's rho; BMI, body mass index; HE4, human epididymis protein 4; CA125, cancer antigen 125.

Table 5. Performance of the markers in predicting the presence of metastases.

Marker	n	Sensitivity	Specificity	PPV	NPV
HE4					
Grade 1	38	75.0	52.9	15.8	94.7
Grade 2	24	66.7	42.9	14.3	90.0
Grade 1+2	62	71.4	49.1	15.2	93.1
Grade 3	29	85.7	31.8	28.6	87.5
All grades	91	78.6	44.2	20.4	91.9
CA125					
Grade 1	38	25.0	97.1	50.0	91.7
Grade 2	24	33.3	90.5	33.3	90.5
Grade 1+2	62	28.6	94.5	40.0	91.2
Grade 3	29	28.6	100	100	81.5
All grades	91	28.6	96.1	57.1	88.1
HE4+CA125[a]					
Grade 1	37[b]	75.0	93.9	60.0	96.9
Grade 2	24	66.7	85.7	40.0	94.7
Grade 1+2	62	71.4	90.7	50.0	96.1
Grade 3	29	71.4	86.4	62.5	90.5
All grades	90	71.4	89.5	55.6	94.4

[a]Using the risk score algorithm for postmenopausal women; [b]one patient younger than 49 years excluded; PPV, positive predictive value; NPV, negative predictive value; HE4, human epididymis protein 4; CA125, cancer antigen 125.