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ULTRASOUND ASSESSMENT AND RISK PREDICTION IN WOMEN WITH ENDOMETRIAL CANCER

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Ultrasound assessment and risk prediction in women with endometrial cancer

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"Life is not measured by the numbers of breaths we take,
but by the moments that take our breath away"

-unknown origin

To my family

ABSTRACT

Background: Endometrial cancer is the most common gynecological cancer in the industrialized world, constituting 4.5% of all cancer in Swedish women. Standard surgery is hysterectomy and bilateral salpingo-oophorectomy, with or without lymphadenectomy, depending on the estimated risk of lymph node metastases. Adjuvant therapy is given, depending on the estimated risk of adverse outcome. Numerous risk stratification systems guide the choice on lymphadenectomy, but none is associated with a high accuracy. Lymph node metastases are found in 11—22% of women. If they could be accurately predicted, many lymphadenectomies could be avoided. The aim of this thesis was to improve preoperative risk assessment in women with endometrial cancer, with regard to predicting deep (≥ 50%) myometrial invasion (MI), cervical stromal invasion (CSI), lymph node metastases and recurrence or progression.

Methods: All study cohorts originate from the prospective, international, multicenter IETA (International Endometrial Tumor Analysis) 4 ultrasound study on women with endometrial cancer. In **Study I** agreement to histopathology and interobserver reproducibility of subjective ultrasound assessment of MI and CSI among ultrasound experts and gynecologists were compared by off-line evaluation of videoclips from 53 women from a single center cohort. In **Study II** sonographic features and accuracy of ultrasound assessment of MI ≥ 50% were compared in tumors with and without the microcystic elongated and fragmented (MELF) pattern of myometrial invasion and the relationship of the MELF pattern to more advanced stage (≥ IB) and lymph node metastases was assessed in 850 women with endometrioid endometrial cancer from a multicenter cohort. In **Study III** a risk prediction model was developed on 1501 women from a multicenter cohort, to estimate the individual risk of lymph node metastases before surgery. In **Study IV** demographic, sonographic and Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) variables and their ability to predict recurrence or progression was assessed in 339 women from a single center cohort.

Results: Gynecologists and ultrasound experts assessed MI ≥ 50% with comparable diagnostic accuracy and interobserver reproducibility, while ultrasound experts assessed CSI with greater diagnostic accuracy and interobserver reproducibility than gynecologists. Tumors with the MELF pattern were slightly larger, the color score was higher and the multiple multifocal vascular pattern was more common, compared to tumors without the MELF pattern. The MELF pattern did not affect the diagnostic accuracy of MI assessment, however it was associated with ≥ 50% MI, CSI, higher stage and lymph node metastases. A risk model with variables from endometrial biopsy results (histotype), clinical (age and bleeding duration) and ultrasound characteristics (tumor extension and tumor size) could reliably predict the risk of lymph node metastases before surgery, and had higher clinical utility than risk stratification by combined endometrial biopsy and ultrasound. Demographic (age ≥ 65 years and waist circumference ≥ 88 cm), sonographic (ultrasound tumor extension and ultrasound AP diameter ≥ 2 cm) and ProMisE variables combined had higher ability to predict recurrence or progression than the ESMO (European Society for Medical Oncology) classification. Ultrasound tumor size < 2 cm and non-p53 abnormal status identified a large group of women (48%) with a very low risk of tumor recurrence or progression (1.8%).

Conclusion: Preoperative ultrasound staging should be performed by ultrasound experts and is not negatively affected by presence of the MELF status. A risk model with variables from endometrial biopsy, clinical and ultrasound characteristics improves preoperative risk prediction of lymph node metastases. Demographic, sonographic and ProMisE variables show the potential to predict tumor recurrence or progression already before surgery.

LIST OF SCIENTIFIC PAPERS

I. Eriksson LSE, Lindqvist PG, Flöter Rådestad A, Dueholm M, Fischerova D, Franchi D, Jokubkiene L, Leone FP, Savelli L, Sladkevicius P, Testa AC, Van Den Bosch T, Ameye L, Epstein E

Transvaginal ultrasound assessment of myometrial and cervical stromal invasion in women with endometrial cancer: interobserver reproducibility among ultrasound experts and gynecologists

Ultrasound Obstet Gynecol 2015;45:476–482

II. Eriksson LSE, Nastic D, Frühauf F, Fischerova D, Nemejcova K, Bono F, Franchi D, Fruscio R, Ghioni M, Haak LA, Hejda V, Meskauskas R, Opolskiene G, Pascual MA, Testa A, Tressera F, Zannoni GF, Carlson JW, Epstein E

Clinical and ultrasound characteristics of the microcystic elongated and fragmented (MELF) pattern in endometrial cancer according to the International Endometrial Tumor Analysis (IETA) criteria

Int J Gynecol Cancer 2019;29:119–126

III. Eriksson LSE, Epstein E, Testa AC, Fischerova D, Valentin L, Sladkevicius P, Franchi D, Frühauf F, Fruscio R, Haak LA, Opolskiene G, Mascilini F, Alcazar JL, Van Holsbeke C, Chiappa V, Bourne T, Lindqvist PG, Van Calster B, Timmerman D, Verbakel JY, Van den Bosch T, Wynants L

An ultrasound-based risk model to predict lymph node metastases before surgery in women with endometrial cancer: a model development study

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IV. Eriksson L SE, Nastic D, Lindqvist PG, Imboden S, Järnbert-Petterson H, Carlson JW, Epstein E

The value of sonographic-, demographic characteristics and the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) to predict tumor recurrence or progression

Manuscript

CONTENTS

LIST OF ABBREVIATIONS

AP	Anteroposterior
AUC	Area under the receiver operating characteristics curve
BMI	Body Mass Index
CI	Confidence Interval
CN	Copy Number
CSI	Cervical Stromal Invasion
CT	Computed Tomography scan
CTNNB1	Catenin Beta 1
ER	Estrogen Receptor
ESGO	European Society of Gynaecological Oncology
ESMO	European Society for Medical Oncology
ESTRO	European Society for Radiotherapy & Oncology
FIGO	International Federation of Gynecology and Obstetrics
GOG	Gynecologic Oncology Group
HER-2/NEU	Human Epidermal Growth Factor Receptor 2
HR	Hazard Ratio
IETA	International Endometrial Tumor Analysis
IOTA	International Ovarian Tumor Analysis
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
LMH1	mutL homolog 1
LVSI	Lymphovascular Space Invasion
MAR	Missing At Random
MCAR	Missing Completely At Random
MELF	Microcystic Elongated and Fragmented
MI	Myometrial Invasion
MMR-D	Mismatch Repair proteins Deficiency
MNAR	Missing Not At Random
MRI	Magnetic Resonance Imaging
MSI	Microsatellite Instability
NPV	Negative Predictive Value
OR	Odds Ratio
p53 wt	protein 53 wild type
p53 abn	protein 53 abnormal
PET	Positron Emission Tomography scan
PIC3CA	Phosphatidylinositol-4.5-bisphosphate 3-kinase

POLE EDM	Polymerase-ε Exonuclease Domain Mutations
PORTEC	Post Operative Radiation Therapy in Endometrial Carcinoma
PPV	Positive Predictive Value
ProMisE	Proactive Molecular Risk Classifier for Endometrial Cancer
PTEN	Phosphatase and Tensin Homologue
RCT	Randomized Controlled Trial
ROC	Receiver Operating Characteristic
SEPAL	Survival Effect of Para-Aortic Lymphadenectomy in Endometrial Cancer
TCGA	The Cancer Genome Atlas
US	United States of America

1 INTRODUCTION

1.1 BACKGROUND

Endometrial cancer is the most common gynecological cancer in the industrialized world, with approximately 1400 new cases in Sweden every year, constituting 4.5% of all cancer in women[1]. The main risk factor for developing endometrial cancer is exposure to estrogens, associated with obesity, early menarche, late menopause and nulliparity, as well as older age, the use of Tamoxifen and hereditary factors[2, 3], whereas oral-contraceptive use and physical activity decrease the risk[3]. Overweight alone is responsible for around half of the cases of endometrial cancer in Europe and the US[4].

Due to early clinical symptoms with bleeding, endometrial cancer is often diagnosed in an early stage with favorable outcomes and a 5-year relative survival of 84%[1]. Diagnosis is obtained through endometrial biopsy from simple biopsy, dilatation and curettage or hysteroscopic resection. Established prognostic factors are FIGO (International Federation of Gynecology and Obstetrics) stage, histotype, FIGO grade, tumor diameter, deep myometrial invasion (≥ 50%), LVSI (lymphovascular space invasion), DNA ploidy, S-phase fraction, age, estrogen receptor (ER) and progesterone receptor (PR) expression and comorbidities[2, 3, 5-8]. FIGO stage is the most important prognostic factor and reflects the 5-year survival of 90% for stage I, 78% for stage II, 62% for stage III and 21% for stage IV[7]. Within a given stage, those who are 80 years or older have a considerably worse prognosis, which to a certain degree may relate to lack of surgical staging and less aggressive adjuvant therapy[7]. Non-endometrioid cancer carry a worse prognosis than endometrioid cancer and though they are more uncommon, they account for more than 50% of recurrences and deaths from endometrial cancer[3]. Five-year overall survival (all stages) are 85% for endometrioid tumors, compared with 62% for clear cell and 53% for serous cancer, with worse survival in non-endometrioid tumors also within a given stage[7]. Though all considered high-risk cancers, serous and clear cell cancers have a significantly poorer prognosis compared with endometrioid cancer grade 3[9]. Five-year overall survival in stage I endometrioid endometrial cancer decreases with increasing grade (grade 1: 93%, grade 2: 90% and grade 3: 79%)[7]. Tumor size and tumor extension are predictive of lymph node metastases[5, 6, 10-25], recurrence and survival[6-8, 11].

Standard surgery in endometrial cancer is hysterectomy and bilateral salpingo-oophorectomy, with or without lymphadenectomy. Sentinel node biopsy has emerged as an alternative to lymphadenectomy, rendering staging information while reducing operation time and morbidity[26]. In the absence of sentinel node biopsy, the choice on lymphadenectomy is guided through preoperative risk stratification, most often based on the tentative stage, histotype and grade[27]. The prevalence of lymph node metastases in endometrial cancer is 11—22%[28-30]. If the risk of lymph node metastases could be better predicted, many lymphadenectomies could be avoided.

1.2 STAGING IN ENDOMETRIAL CANCER

The main goal of staging is to standardize management and to allow comparison of therapeutic strategies. The FIGO staging[31] and the TNM classification[32] are the staging systems most in use[2].

The first FIGO staging for endometrial cancer was adopted in 1950 and was clinical, meaning that the spread of the tumor was defined by clinical examination (Table 1). Staging was done after a determination of whether the cancer was confined to the corpus or had spread beyond it. Subdivision was performed depending on whether the woman was medically operable or not. However, women with clinical stage I disease where found to have tumor spread beyond the uterus, and an association between tumor grade, depth of myometrial invasion, extension to cervix and/or adnexa and the risk of lymph node metastases was demonstrated[5, 33]. Since 1988 endometrial cancer is surgically staged. The major changes in the 1988 staging system were the use of the depth of myometrial invasion, the identification of tumor cells in peritoneal cytologic examination and the addition of lymph node metastases status, making lymphadenectomy necessary to obtain a complete surgical staging[34].

A 2017 Cochrane review[35] based on two randomized controlled trials (RCT); Benedetti-Panici et al[36] and the ASTEC trial[37], found no evidence that lymphadenectomy decreases the risk of death (overall survival: pooled hazard ratio (HZ) 1.07, 95% confidence interval (CI) 0.81—1.43) or disease recurrence (recurrence-free survival: pooled HR 1.23, 95% CI 0.96—1.58) compared with no lymphadenectomy, in women with presumed stage I disease (1851 participants, two studies, moderate-quality evidence). It also concluded that women who were subject to lymphadenectomy were more likely to experience surgery-related systemic morbidity (RR 3.72, 95 % CI 1.04—13.27) or lymphedema/lymphocyst formation (RR 8.39, 95 % CI 4.06—17.33), than those who did not undergo lymphadenectomy (1922 participants, two studies, high quality evidence). This has formed the rationale of not performing lymphadenectomy in so-called low-risk cases. The extent of surgical staging also varies depending on patient age, comorbidities and local practice.

The including studies have been criticized in the retrospective SEPAL study[38] for a short follow-up period (median 37 months, with 35.7% of surviving patients followed-up for less than 3 years)[37], selective rather than systematic lymphadenectomy (removal of 9 or fever lymph nodes in 35% of women in the lymphadenectomy group)[37], for not including para-aortic lymphadenectomy[36, 37] and for not assessing risk of recurrence[36]. The SEPAL study also reported a restricted survival effect of lymphadenectomy in low-risk women but a substantial therapeutic effect in women with intermediate- or high risk, with longer overall-, disease-specific- and recurrence-free survival in women subject to combined pelvic and para-aortic lymphadenectomy compared to pelvic lymphadenectomy only[38].

No RCT evidence shows the effect of lymphadenectomy in women at high risk of recurrence or higher-stage disease[35].

Table 1. Staging of endometrial cancer according to FIGO over time[31,34]

Clinical staging	1950 – 1961	
	Stage 0	Cases which the pathologist considers most likely to be of a carcinomatous nature though it is impossible to arrive at a definite microscopic diagnosis
	Stage I	The growth is confined to the uterus
	Group 1	Operation advisable
	Group 2	Bad operative risks
	Stage II	The growth has spread outside the uterus
	1962 – 1971	
	Stage 0	Histological findings suspicious of malignancy but not proven
	Stage I	The carcinoma is confined to the corpus
	Stage II	The carcinoma has involved the corpus and the cervix
	Stage III	The carcinoma has extended outside the uterus but not outside the true pelvis
	Stage IV	The carcinoma has extended outside the true pelvis or has obviously involved the mucosa of the bladder or rectum
	1971 – 1988	
	Stage 0	Carcinoma in-situ. Histological findings suspicious of malignancy
	Stage I	The carcinoma is confined to the corpus
	IA	The length of the uterine cavity is 8 cm or less
	IB	The length of the uterine cavity is greater than 8 cm
	Stage II	The carcinoma has involved the corpus and the cervix
	Stage III	The carcinoma has extended outside the uterus but not outside the true pelvis
	Stage IV	The carcinoma has extended outside the true pelvis or has obviously involved the mucosa of the bladder or rectum
Surgical staging	1988 – 2009	
	Stage I	Tumor limited to the corpus
	IA	Endometrium only
	IB	Invasion < 50% of the myometrium
	IC	invasion ≥ 50% of the myometrium
	Stage II	Involvement of the cervix
	IIA	Cervical glandular invasion only
	IIB	Cervical stromal invasion
	Stage III	Spread outside of the uterus, confined to pelvis (not including bladder or rectal involvement)
	IIIA	Involvement of the uterine serosa, adnexa or positive peritoneal cytology
	IIIB	Spread to vagina
	IIIC	Pelvic or para-aortic lymph node metastases
	Stage IV	Spread to the bladder, rectum, distant sites
	IVA	Involvement of bladder and/or rectal mucosa
	IVB	Distant, intra-abdominal spread, inguinal lymph node metastases
	2009 –	
	Stage I	Tumor confined to the corpus uteri
	IA	No or < 50% myometrial invasion
	IB	Myometrial invasion ≥ 50%
	Stage II	Cervical stromal invasion
	Stage III	Tumor with local and/or regional extension
	IIIA	Tumor invades the serosa of the corpus uteri and/or adnexa
	IIIB	Vaginal and/or parametrial involvement
	IIIC	Metastases to pelvic and/or para-aortic lymph nodes
	IIIC1	Pelvic lymph node metastases
	IIIC2	Para-aortic lymph node metastases, with or without pelvic lymph node metastases
	Stage IV	Tumor involving bladder and/or bowel mucosa, and /or distant metastases
	IVA	Tumor invasion of bladder and/or bowel mucosa
	IVB	Distant disease, including intra-abdominal metastases and/or inguinal lymph node metastases

1.3 IMAGING IN ENDOMETRIAL CANCER

Imaging is used in the preoperative work-up to guide primary treatment. Computed tomography (CT) scan or positron emission tomography (PET) scan is used to assess extrauterine spread, whereas transvaginal ultrasound or magnetic resonance imaging (MRI) is used to assess deep myometrial invasion, presence of cervical stromal invasion (CSI) and to rule out ovarian disease[27].

1.3.1 Ultrasound

Preoperative ultrasound examination in women with endometrial cancer should be performed systematically, using a combination of transvaginal- and transabdominal ultrasound. Examinations should be performed with the woman in the lithotomy position with an empty bladder, and render information on tumor location, tumor extension and size, morphology and vascularization. A high-end ultrasound system should be used, with a two-dimensional or three-dimensional 3—5 to 9—10 MHz transvaginal transducer and a convex array abdominal probe (3.5—7 MHz). The transvaginal approach is optimal for examination of the uterus. If transvaginal examination cannot be performed, the probe can be inserted transrectally. Scanning of the uterus should be performed in the sagittal plane, from cornu to cornu, and in the transverse plane, from the cervix to the fundus, to establish an overview.

In order to assess cervical stromal invasion the ultrasound image should be magnified in the sagittal plane to contain only the cervix uteri. The level of entry of the uterine arteries corresponds approximately to the internal cervical os. The assessment of stromal invasion can be aided by the application of pressure with the transvaginal probe, to see if the tumor slides against the endocervical mucosa. In the case of gross cervical invasion, parametrial invasion should be assessed. If the tumor has its greatest dimensions in the isthmus region, it can be difficult to determine if the primarity of the tumor is the endometrium or the cervix, and tru-cut biopsy can be diagnostic.

After the cervix has been assessed, the whole uterine body should be visualized, with magnification of the image in the sagittal plane to contain only the uterine corpus. Scanning should be performed in the sagittal plane, from cornu to cornu, and in the transverse plane, from the cervix to the fundus, with description of myometrial invasion and endometrial morphology using grayscale ultrasound, and of vascularization using color and power Doppler. When using color and power Doppler, the Doppler box should include the endometrium and the surrounding myometrium and the settings should be adjusted to an ultrasound frequency of at least 5 MHz, pulse repetition frequency 0.3—0.9 kHz, and reduction of the power Doppler gain until all color artifacts disappear.

Extrauterine tumor extension should be described, using both transvaginal- and transabdominal ultrasound[39-41].

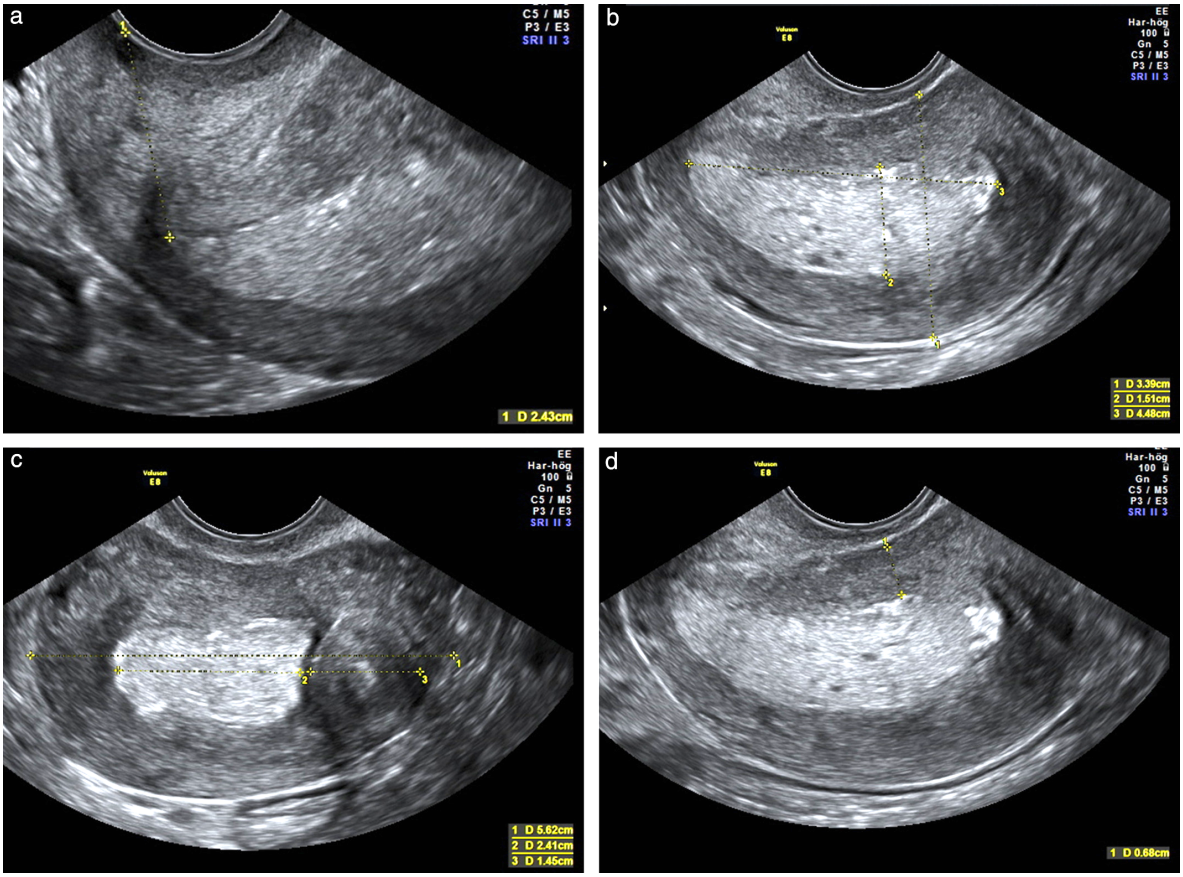

Figure 1. *Ultrasound tumor measurements: a) the distance from the lower margin of the tumor to the outer cervical os in the sagittal plane, b) the anteroposterior (AP) uterine diameter, the AP tumor diameter (tumor thickness) and the craniocaudal tumor diameter (tumor length) in the sagittal plane, c) laterolateral uterine diameter (uterine width) and laterolateral tumor diameter (tumor width) in the transverse plane and d) minimal tumor-free margin to the serosa, measurable in any plane. Reprinted with permision*[66]

1.3.1.1 Staging

Deep myometrial invasion can be evaluated using subjective assessment, by estimating the proportion of tumor invasion in relationship to the normal myometrial wall thickness (< 50% or ≥ 50%) (Figure 2), or by measurement techniques, such as the deepest invasion/normal myometrium ratio (Gordon's ratio)[42], tumor/uterine anteroposterior (AP) diameter ratio, (Karlsson's ratio)[43], endometrial thickness[44], tumor/uterine volume ratio[44], minimal tumor-free margin/uterine AP diameter ratio[44] and the shortest distance to serosa[45] (Figure 1).

Cervical stromal invasion can be evaluated using subjective assessment (Figure 2) or by measurement techniques, measuring the distance from the lower margin of the tumor to the outer cervical os[44] (Figure 1).

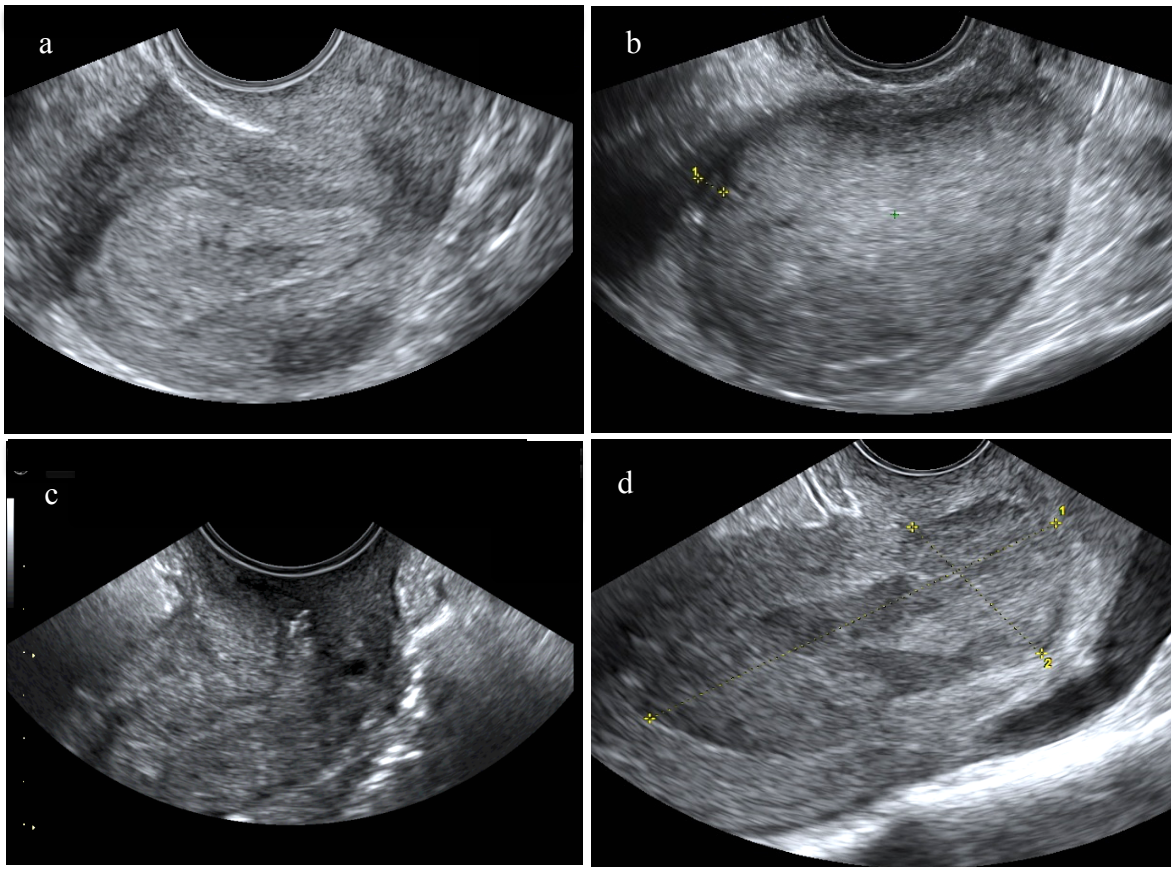

***Figure 2**. Ultrasound tumor extension: a) myometrial invasion < 50%, b) myometrial invasion ≥ 50%, c) no cervical stromal invasion, d) cervical stromal invasion.*

Subjective assessment of deep myometrial invasion and cervical stromal invasion performs better than measurement techniques and is associated with sensitivities and specificities ranging from 61%—93% vs. 71%—90%, respectively, for deep myometrial invasion[44-56] and 29%—93% vs. 85%—99%, respectively, for cervical stromal invasion[44, 48, 52, 55-62] (Table 2). The accuracy of subjective assessment of deep myometrial invasion and cervical stromal invasion is affected by tumor size and tumor vascularity, with increased risk of underestimation in tumors with lower volume, lesser endometrial thickness, thicker minimum tumor-free myometrium and lower color score (1—2) and increased risk of overestimation in tumors with higher volume, greater endometrial thickness, lesser minimum tumor-free myometrium and higher color score (3—4). BMI, uterine position and image quality has not been shown to affect the risk of staging error[63].

The adnexal region should be assessed, to rule out ovarian spread or synchronous ovarian cancer, reported to occur in 5% of cases[64]. Ultrasound-guided tru-cut biopsy can be used to establish tumor primarity in most cases. Adnexal metastases can be diagnosed by ultrasound with a sensitivity of 73% and a specificity of 98%, whereas assessment of pelvic lymph node metastases is associated with poor sensitivity (sensitivity 33% and specificity 100%)[58].

Table 2. Subjective assessment of deep myometrial invasion (≥ 50%) and cervical stromal invasion by transvaginal ultrasound

			Deep myometrial invasion		Cervical stromal invasion	
	Year	N=	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Gabrielli et al.	1996	67	—	—	54	87
van Doorn et al.	2002	93	79	72	—	—
Sawicki et al.	2003	90	—	—	86	85
De Smet et al.	2006	97	61	86	—	—
Savelli et al.	2008	74	84	83	93	92
Alcazar et al.	2009	96	93	82	—	—
Ozdemir et al.	2009	64	85	75	—	—
Celik et al.	2010	64	—	—	88	92
Akbayir et al.	2011	298	—	—	77	99
Akbayir et al.	2012	219	62	81	—	—
Savelli et al.	2012	155	75	89	—	—
Antonsen et al.	2013	195	71	72	29	92
Mascilini et al.	2013	144	77	81	54	93
Ortoft et al.	2013	156	—	—	38	89
Van Holsbeke	2014	211	83	71	—	—
Alcazar et al.	2015	169	80	90	—	—
Christensen et al.	2016	110	—	—	39	88
Frühauf et al.	2017	210	79	73	41	94
Verbakel et al.	2019	1538	70	80	49	94

1.3.1.2 Endometrial morphology

Assessment of endometrial morphology according to IETA (International Endometrial Tumor Analysis) includes description of echogenicity, the endometrial midline, bright edge and the endometrial-myometrial junction[40].

The echogenicity of the endometrium is compared with the echogenicity of the myometrium, and described as "uniform", if the endometrium is homogenous and symmetrical (Figure 3) or "non-uniform", if the endometrium is heterogeneous, asymmetrical or cystic (Figure 4). The endometrial midline is described as "linear" or "non-linear", if a hyperechogenic interface within the endometrium is visualized, and "irregular" or "not defined", in the absence of a distinct interface (Figure 5). If an echo is formed by the interface between an intracavitary lesion and the endometrium it is called the "bright edge" (Figure 6). The border between the endometrium and myometrium, called the endometrial-myometrial junction, is described as "regular", "irregular", "interrupted" or "not defined" (Figure 7).

On ultrasound, non-uniform echogenicity and a non-regular endometrial-myometrial junction are associated with higher grade, higher stage and high-risk cancer[65, 66].

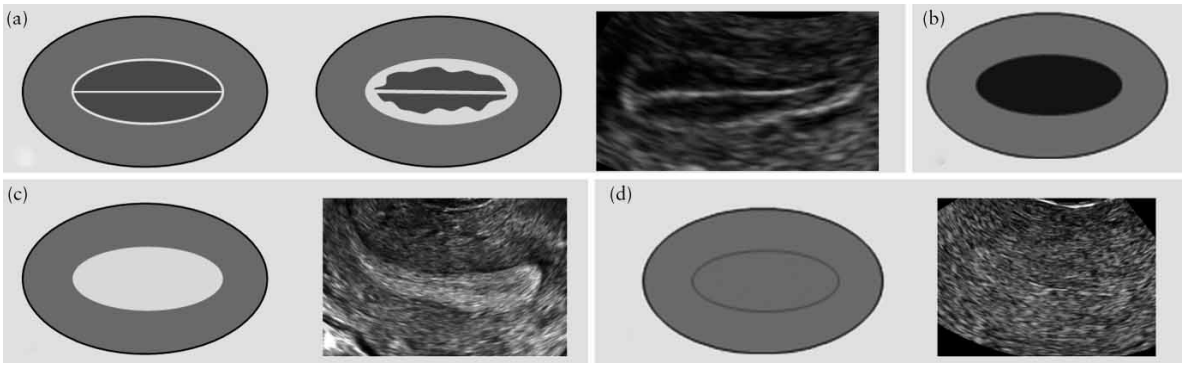

Figure 3. *Uniform echogenicity: a) three-layer pattern, b) hypoechogenic, c) hyperechogenic and d) isoechogenic. Reprinted with permission*[40]

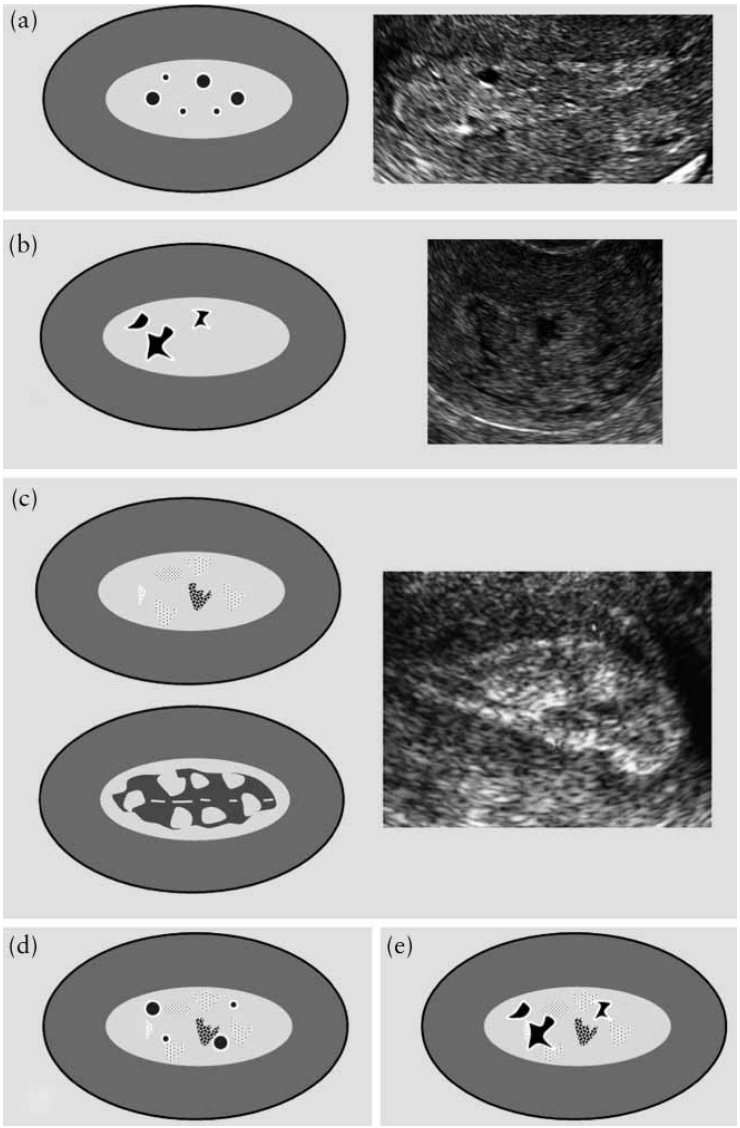

Figure 4. *Non-uniform echogenicity: a) homogeneous background with regular cystic areas, b) homogenous background without irregular cystic areas, c) heterogeneous background without cystic areas, d) heterogeneous background with regular cystic areas and e) heterogeneous background with irregular cystic areas. Reprinted with permission*[40]

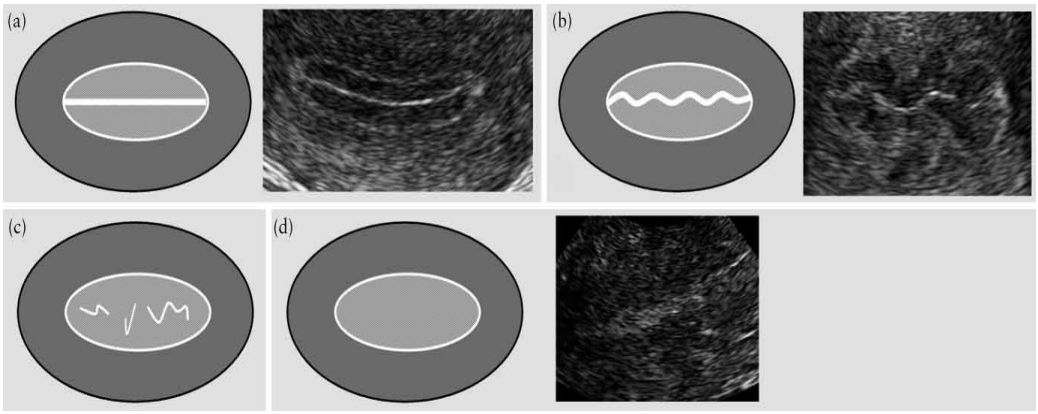

***Figure 5**. Endometrial midline: a) linear, b) non-linear, c) irregular and d) not defined. Reprinted with permission*[40]

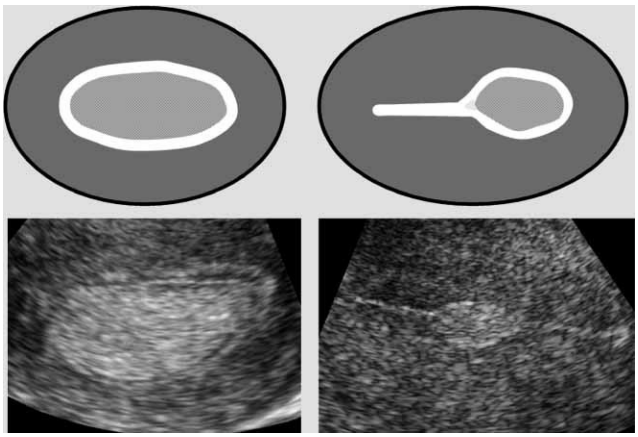

***Figure 6**. Bright edge. Reprinted with permission*[40]

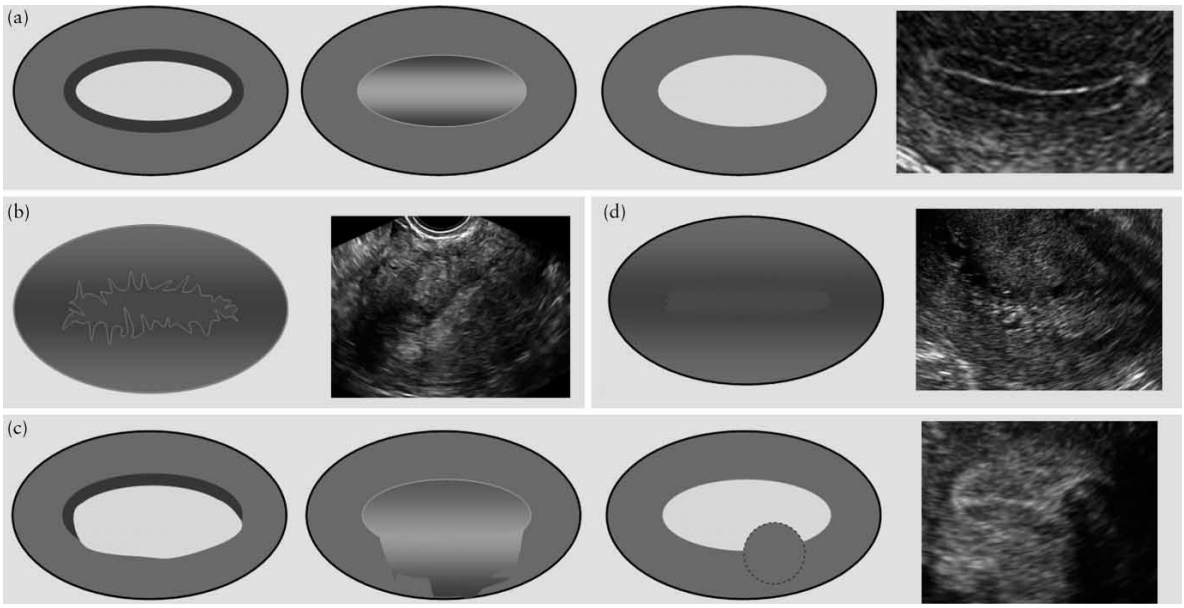

***Figure 7**. Endometrial-myometrial junction: a) regular, b) irregular, c) interrupted and d) not defined. Reprinted with permission*[40]

1.3.1.3 Color score and vascular Morphology using power Doppler

The color score is a subjective semi quantitative assessment of the amount of blood flow present in the endometrium, and may be scored using the International Ovarian Tumor Analysis (IOTA) color score for ovarian masses[67]. The color flow reflects the blood flow, resulting in a color score from 1—4[40] (Figure 8). The vascular pattern describes the distribution of vessels within the endometrium, and is reported with respect to the presence or absence of dominant vessels or other specific patterns[40]. Focus is put on the number of vessels (single or multiple) and the myometrial-endometrial junction origin of the vessels (focal, multifocal or not visible) (Figure 9). On ultrasound, multiple vessels of focal or multifocal origin and a higher color score are associated with endometrioid grade 3 or non-endometrioid tumors, larger tumor size, higher stage and high-risk cancer[65, 66].

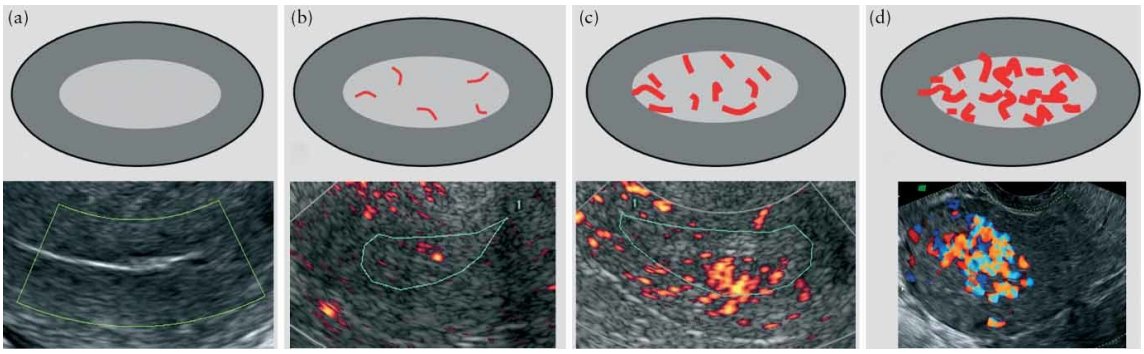

Figure 8. *Color score: a) color score 1: no color flow, b) color score 2: minimal color flow, c) color score 3: moderate color flow, d) color score 4: abundant color flow. Reprinted with permission*[40]

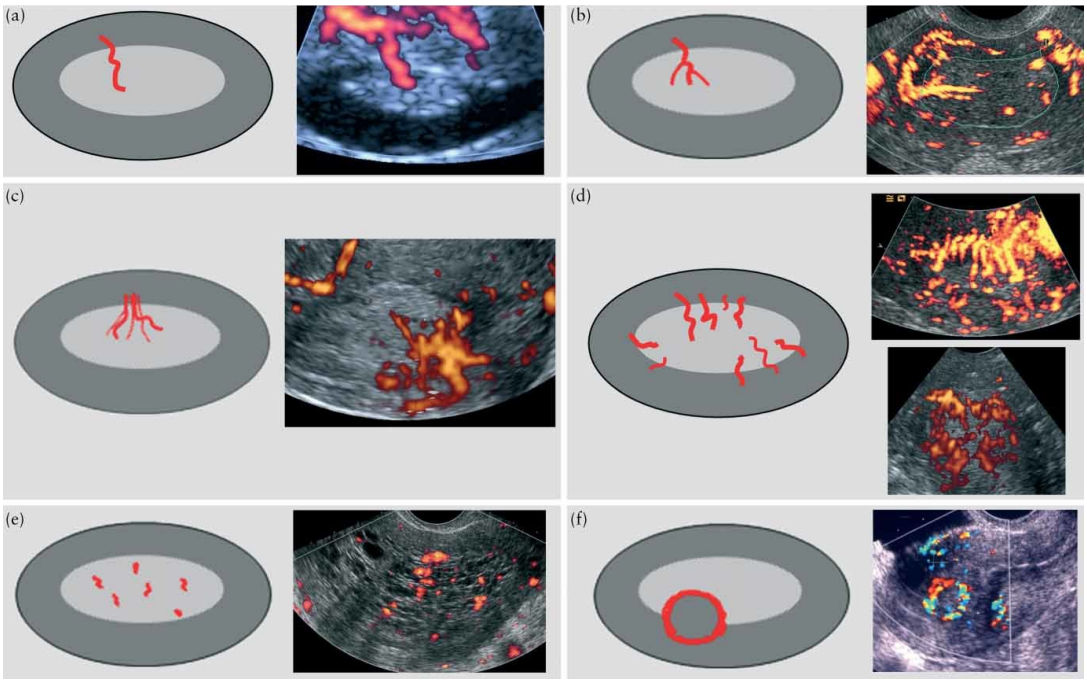

Figure 9. *Vascular pattern: a) single dominant vessel without branching, b) single dominant vessel with branching, c) multiple vessels with focal origin, d) multiple vessels with multifocal origin, e) scattered vessels and f) circular flow. Reprinted with permission*[40]

1.3.1.4 Tumor size

The size of the tumor is measured using three perpendicular diameters; tumor AP diameter (when the tumor is defined) or endometrial thickness (when the tumor is not defined) in the sagittal plane, longitudinal diameter in the sagittal plane and tumor lateral diameter in the transverse plane. The relative tumor size is measured through the AP tumor/uterine diameter ratio. The tumor volume is obtained either from 2D ultrasound using the approximate formula for an ellipsoid, ((AP diameter × length × width) / 2)[66] or from 3D ultrasound measurement[68] (Figure 1).

Increased tumor size, as measured by tumor volume or endometrial thickness, is associated with deep myometrial invasion, non-endometrioid cancer, higher grade, higher stage, lymph node metastases and high-risk cancer[66, 69].

1.3.1.5 Three-dimensional (3D) ultrasound

Three-dimensional ultrasound enables rapid acquisition of ultrasound images, with the ability to display volume-rendered images of the uterine corpus and the cervix. Three-dimensional ultrasound allows virtual navigation through multiplanar display, with off-line analysis in any plane, including the reconstructed coronal plane, and surface rendering, i.e. the reconstruction of the outline and the internal contour of the uterus.

Three-dimensional ultrasound enables calculation of vascular indexes (vascularization index, flow index and vascularization-flow index), a 3D reconstruction of the vascular tree and the calculation of tumor volumes even in irregularly shaped structures. In 3D ultrasound, the volume contrast imaging technique projects a 1-10 mm slice of a volume dataset onto a two-dimensional (2D) screen. The obtained image has an enhanced tissue demarcation, due to the filling of gaps in the image by tissue information from adjacent layers.

Due to computer generation, 3D ultrasound is sensitive to motion artifacts and pre-acquisition image quality and requires more user input than 2D imaging, as data must be manipulated after being obtained. An inappropriate technique when manipulating data can result in the creation of artifacts[68, 70].

Three-dimensional ultrasound has been associated with reliable assessment of myometrial invasion[45] but has not proved superior to 2D ultrasound[62] or MRI[62, 71, 72] in the assessment of deep myometrial invasion[62, 71, 72] or cervical stromal invasion[71].

1.3.1.6 Ultrasound appearance of endometrial cancer

Grayscale and color power Doppler sonographic features in endometrial cancer vary with histotype, stage and grade. With increasing stage and grade, tumors are larger, have higher color score and are less likely to have regular endometrial-myometrial junction or uniform echogenicity. Non-endometrioid tumors are generally larger than endometrioid tumors, with a vascularity similar to that of high-grade (grade 3) endometrioid tumors but a morphology similar to that of low-grade (grade 1—2) endometrioid tumors.

Compared to low-grade tumors and low-risk tumors (low-grade endometrioid cancer stage IA), high-grade tumors and high-risk tumors (endometrioid cancer grade 3, non-endometrioid cancer or stage ≥IB) have greater size, as measured by endometrial thickness and tumor volume, are less likely to have uniform echogenicity or regular endometrial-myometrial junction and more likely to have multiple vessels of focal or multifocal origin and a higher color score[66] (Figure 10).

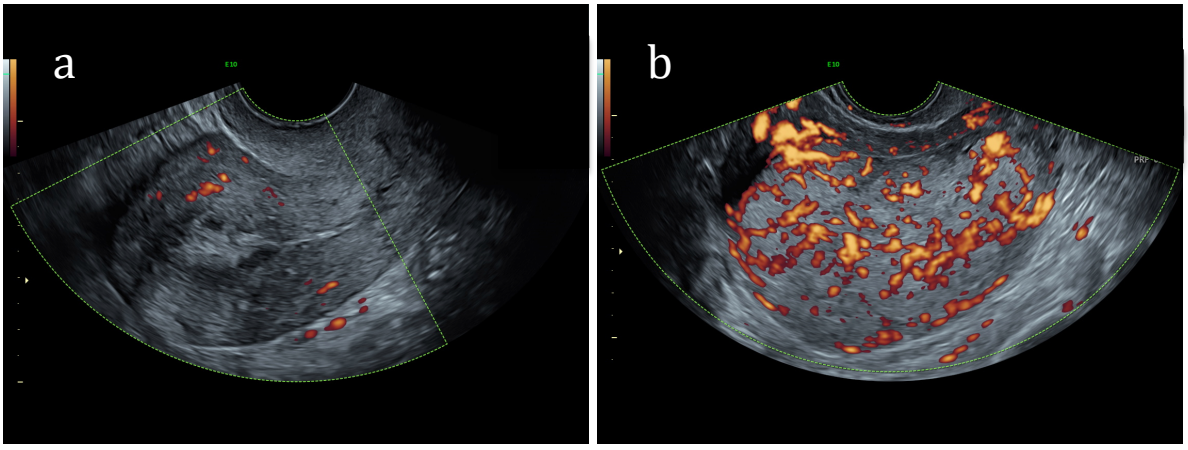

***Figure 10**. Tumor ultrasound appearance depending on risk: a) low-risk tumor, b) high-risk tumor.*

1.3.1.7 Strengths and limitations of ultrasound

Ultrasound poses the advantages of wide availability, low cost, short examination time, no ionizing radiation and no need for intravenous contrast, but requires a skilled examiner with a high constant rate of examinations to maintain skill. The possibility to assess the endometrium by transvaginal ultrasound is limited if the uterus is in the upright position, which may be the case in obese women due to excess intra-abdominal fat[39]. In these cases, the addition of transabdominal or even transrectal ultrasound may give additional information. Still, in the minority of cases image-quality is too poor to delineate the tumor using ultrasound, and MRI is an alternative, unless there are contraindications to MRI as well.

1.3.2 MRI

Meta-analyzes on assessment of deep myometrial invasion[73-76] and cervical stromal invasion[75, 76] by MRI report sensitivities and specificities ranging from 79%—90% vs. 81%—95% for deep myometrial invasion and 50%—57% vs. 95% for cervical stromal invasion.

Publications comparing ultrasound and MRI in the same set of women show similar results regarding the assessment of deep myometrial invasion[48, 49, 52] and cervical stromal invasion[48, 52, 59, 61, 62, 77] (Table 3). In accordance to ultrasound, MRI has a low sensitivity for the prediction of lymph node metastases (sensitivity 59% and specificity 95%)[76].

Table 3. Comparison of transvaginal ultrasound and magnetic resonance imaging (MRI) in the subjective assessment of deep myometrial invasion (≥ 50%) and cervical stromal invasion in the same set of women

a. Deep myometrial invasion

			Ultrasound		MRI	
	Year	N=	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Cicinelli et al	2008	100	—	—	—	—
Savelli et al	2008	74	84	83	84	81
Ozdemir et al	2009	64	85	75	85	79
Celik et al	2010	64	—	—	—	—
Antonsen et al	2013	123	69	74	89	57
Ortoft et al	2013	156	—	—	—	—
Christensen et al	2016	110	—	—	—	—

b.Cervical stromal invasion

			Ultrasound		MRI	
	Year	N=	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Cicinelli et al	2008	100	53	82	67	95
Savelli et al	2008	74	93	92	79	87
Ozdemir et al	2009	64	—	—	—	—
Celik et al	2010	64	88	92	88	94
Antonsen et al	2013	123	19	94	27	94
Ortoft et al	2013	156	38	89	54	91
Christensen et al	2016	110	39	88	56	90

1.4 HISTOPATHOLOGY IN ENDOMETRIAL CANCER

Histopathological assessment plays a central role in endometrial cancer, by classifying the tumor and by staging it. Endometrial cancer is classified on the basis of tumor cell type and, in the case of endometrioid cancer, also by grade[78].

1.4.1 Cell type

Endometrial cancers are classified as endometrioid (80—90%) or non-endometrioid (10—20%), including various subtypes such as serous, clear-cell, undifferentiated carcinoma or carcinosarcoma[27]. Assessment of cell type is made on the basis of histological criteria on hematoxylin and eosin (H&E) stain and is critical, as it determines the extent of surgery and the use of adjuvant therapy. The main categories are the endometrioid adenocarcinoma, which contains glands resembling those of the normal endometrium, and is often found on a background of atypical hyperplasia, the serous carcinoma, which is characterized by a complex pattern of papillae with cellular budding, and is often found on a background of atrophical endometrium, the clear cell adenocarcinoma, which is composed mainly of clear and hobnail cells, in solid tubulocystic or papillary patterns and the undifferentiated carcinomas, which lack any evidence of differentiation. Also, mixed tumors are common.

Sometimes, differential diagnostics for cell type can be difficult, for instance between endometrioid and serous carcinoma. Immunohistochemistry can be used as a diagnostic aid, based on immunophenotypic differences between cell types. A panel of immunomarkers, containing p53, estrogen receptor (ER), phosphatase and tensin homologue (PTEN) and p16 are often used to distinguish endometrioid (p53 negative, ER positive, PTEN negative, p16 negative) from serous carcinoma (p53 positive, ER negative, PTEN positive, p16 positive). Interpretative difficulties remain in cases with intermediate immunostaining results[78].

1.4.2 Grade

According to the 1988 FIGO three-tire grading system, the tumor is graded based on the percentage of solid non-squamous growth; grade 1: ≤ 5% solid growth; grade 2: 6—50% solid growth; grade 3: > 50% solid growth. If there is notable nuclear atypia in grade 1 or grade 2 tumors, the grade is increased by one[78, 79].

Problems with the FIGO grading are difficulties in distinction between solid growth and areas of immature squamous metaplasia or compressed confluent glands, quantification of the percentage of solid growth near cut-off points between grades and lacking morphological and morphometric criteria on notable nuclear atypia. These interpretive difficulties confer a limited interobserver reproducibility for grade. Also, cut-offs between grades have been arbitrary set, and not driven based on outcome[78].

Two-tired (binary) grading systems have been suggested[80-83], to improve reproducibility and to make grading more compatible with treatment options with two alternatives, such as to perform lymphadenectomy or not and to give adjuvant treatment or not. In contrast to the two-tired grading system in serous carcinoma of the ovary, where there are fundamental differences, including molecular differences (p53 and Ki-67), between low- and high grade, endometrioid endometrial cancer exhibits a continuum between low- and high grade, without any reliable molecular markers to aid in the development of a binary grading system[78].

1.4.3 Stage

The surgical specimen is assessed regarding the depth of myometrial invasion (< 50% vs. ≥ 50%), the presence of cervical stromal invasion (no vs. yes) and extrauterine spread (no vs. yes, as well as location of spread, if present), to determine the surgical stage according to the FIGO 2009 staging system[31].

The depth of myometrial invasion is measured at the point of deepest invasion and affects tumor stage. Instead of conventional myometrial invasion, a subset of endometrioid endometrial cancers exhibit the microcystic elongated and fragmented (MELF) pattern of myometrial invasion[78]. The MELF pattern shows distinctive morphologic alterations, with detached neoplastic glands, appearing either as microcysts (M), elongated structures (EL) or fragmented small solid clusters or single cells (F)[84] and can be found in 7—48% of endometrioid endometrial cancer[84-92] (Figure 11). Separation of epithelial formations from the superficial, more easily identified tumor from which they originated can lead to an

underestimation of the deepest extent of myometrial invasion, with subsequent tumor understaging [84-87, 92]. In addition, the MELF pattern has been suggested to represent a more aggressive variant of endometrioid endometrial cancer, as it has been associated with lymph node metastases [85-90, 93] and an increased risk of recurrence [94].

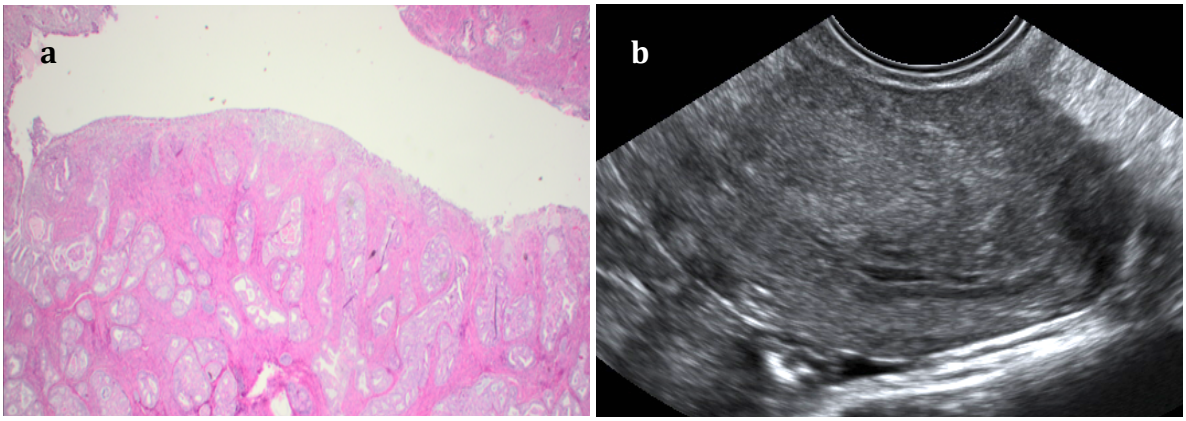

***Figure 11**. The microcystic elongated and fragmented (MELF) pattern of myometrial invasion, on a) histopathology and b) ultrasound.*

1.5 INTEROBSERVER REPRODUCIBILITY IN ENDOMETRIAL CANCER

High interobserver agreement or reproducibility, i.e. how well different assessors agree on the interpretation, is crucial for the usefulness of a diagnostic test.

1.5.1 Ultrasound

When study I was designed, published interobserver reproducibility studies on ultrasound in endometrial cancer were limited to 3D ultrasound in relation to endometrial volume and vascularity measurements[95] and there were no publications on interobserver reproducibility in ultrasound staging.

1.5.2 MRI

Interobserver reproducibility in MRI staging (1.5 T MRI) has been reported to be fair to good for deep myometrial invasion (kappa (κ) 0.32—0.67) and moderate to good for cervical stromal invasion (κ 0.50—0.76) and lymph node metastases (κ 0.54—0.74)[96-98].

1.5.3 Histopathology

Interobserver reproducibility in histopathological staging has been reported to be good to very good for deep myometrial invasion (κ 0.75—0.84) and moderate for cervical stromal invasion (κ 0.49)[99-101]

Tumor histotype and grade have limited interobserver reproducibility (κ 0.62—0.87 vs. 0.35—0.65, respectively)[78, 102], especially in high-grade tumors, as defined by grade 3 endometrioid cancer or non-endometrioid cancer[103, 104]. Binary grading systems have been

associated with higher interobserver reproducibility than the three-tire FIGO grading system[80, 81, 83, 102, 105, 106]. Converting the three-tire FIGO grading system into a two-tire grading system by combining grade 1 and 2 has been associated with prognostic significance for locoregional and distant recurrence and disease-specific survival and to be associated with a higher interobserver reproducibility[78, 82, 83, 106].

A meta-analysis from 2017 (45 studies, 12 459 women) on agreement between preoperative biopsy and final histology according to the hysterectomy specimen reported a pooled agreement of 67% for grade, with the lowest agreement in preoperative grade 2 cancer (61%). Agreement on histologic subtype was 95% for endometrioid cancer and 81% for non-endometrioid cancer. Authors concluded that overall, agreement on tumor grade between preoperative biopsy and the hysterectomy specimen was only moderate[107].

Histopathologic morphology assessment has been reported to only identify two different prognostic groups reproducibly, i.e. high-grade and low-grade tumors, and authors concluded that a more refined risk assessment in endometrial cancer requires the use of molecular markers[106].

1.6 MOLECULAR MARKERS IN ENDOMETRIAL CANCER

Several molecular markers have been identified for endometrial cancer, constituting hormone receptors, DNA ploidy, oncogenes, cancer suppressor genes, mismatch repair genes, apoptosis-associated genes and indicators of cell proliferation[108]. Molecular markers offer the advantage of objective results, as they are based on the presence or the absence of a protein or mutation. They have been used in endometrial cancer to improve sub classification[109-115], to detect prognostic subgroups[116-122], in risk prediction models[123-126] and in individualized therapy[127].

Historically, endometrial cancer was classified into two pathogenic types: type I, comprising endometrioid endometrial cancer associated with good prognosis and type II, comprising non-endometrioid endometrial cancer associated with a higher risk of metastases and poor prognosis[128]. Molecular data have supported this dichotomous classification, with genetic alterations in PTEN, KRAS2, CTNNB1 and PIK3CA and LMH1 promoter hypermethylation in type I cancer[27] and mutations in p53 and HER-2/neu in type II cancer[3]. However, considerable molecular heterogeneity exists, with expression of p53 mutations in 25% of endometrioid grade 3 cancer[129]. Also, its prognostic value is limited, as 20% of type I cancer relapse, whereas 50% of type II cancers do not[2].

The Cancer Genome Atlas (TCGA) Research Network developed a genomic classification of endometrial cancer into four prognostic subgroups: POLE ultramutated, microsatellite instability hypermutated (MSI), copy-number low (CN low) and copy-number high (CN high)[129]. The TCGA classification is associated with complex and costly methodologies, and the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) was developed and validated as a clinically applicable surrogate molecular classification system[130-133]. It is based on a combination of mutation and protein expression analysis and renders four distinct

prognostic subgroups: polymerase-ε exonuclease domain mutations (POLE EDM), mismatch repair proteins deficiency (MMR-D), protein 53 wild type (p53 wt) and protein 53 abnormal (p53 abn). The level of consensus of histotype and grade assessment has been shown to vary with ProMisE subtype (p53 wt: 90%, POLE EDM: 65%, MMR-D: 58% and p53 abn: 39%)[134] and compared to histotype and grade, ProMisE is associated with a higher agreement between preoperative biopsy and hysterectomy specimen[133, 135, 136].

1.7 RISK ASSESSMENT IN ENDOMETRIAL CANCER

There is no universal definition of "low risk" and "high risk" in endometrial cancer. Numerous risk stratification systems are used for classifying the risk of lymph node metastases and recurrence[2, 137, 138], such as the Mayo criteria[6], PORTEC-1[139] (Post Operative Radiation Therapy in Endometrial Carcinoma), GOG-99[140] (Gyneacologic Oncology Group adjuvant radiation for intermediate-risk endometrial cancers), SEPAL[38] (Survival Effect of Para-Aortic Lymphadenectomy in endometrial cancer), ESMO classification[141] (European Society of Medical Oncology), ESMO modified classification[142] and Mayo-modified criteria[143] (Table 4). When compared, the ESMO modified classification most accurately predicted lymph node metastases[138, 144] and risk of recurrence[144].

Published preoperative risk models in endometrial cancer predict the risk of lymph node metastases[25, 145-149] (Table 5), postoperative high risk[53, 61, 123] and advanced stage[150], with[25, 53, 61, 145-149] or without[123, 150] the use of imaging. Some preoperative risk models for lymph node metastases[145-147] have been subject to external validation, limited by selective cohorts and missing data, due to the retrospective nature of the studies[151, 152]. Comparison of model performance is difficult, as long as the models are not validated on the same identical validation cohort, representative to the target population for the model. Tumor histotype and grade have limited interobserver reproducibility[78, 102-104] and agreement on tumor grade between preoperative biopsy and the hysterectomy specimen is only moderate[107], limiting their use in preoperative risk classification. Possibly could molecular classification systems allow more objective results, as they are based on the presence or the absence of a protein or a mutation. Integration of histopathologic and molecular factors has been shown to improve risk assessment in women with early-stage endometrial cancer[120].

Table 4. Classification systems used in endometrial cancer

Classification	Year	N =	Variables
Mayo criteria[6]	2000	328	Histotype (endometrioid) FIGO grade (1 vs. 2 vs. 3) Myometrial invasion (no vs. ≤ 50% vs. > 50%) Primary tumor diameter (≤ 2 cm vs. > 2 cm)
PORTEC-1[139]	2000	715	Age Histotype (endometrioid vs. non-endometrioid) FIGO grade (1 vs. 2 vs. 3) Myometrial invasion (< 50% vs. ≥ 50%)
GOG-99[140]	2004	392	Age Histologic type (endometrioid vs. non-endometrioid) FIGO grade (1 vs. 2 vs. 3) Myometrial invasion (< outer 1/3 vs. ≥ outer 1/3) FIGO stage LVSI
SEPAL[38]	2010	671	Histologic type (endometrioid vs. non-endometrioid) FIGO grade (1 vs. 2 vs. 3) FIGO stage LVSI
ESMO[141]	2013	—	Histologic type (endometrioid vs. non-endometrioid) FIGO grade (1 vs. 2 vs. 3) Myometrial invasion (< 50% vs. ≥ 50%)
ESMO modified[142]	2014	496	Histologic type (endometrioid vs. non-endometrioid) FIGO grade (1 vs. 2 vs. 3) Myometrial invasion (< 50% vs. ≥ 50%) LVSI
Mayo-modified criteria[143]	2014	19 329	Histotype (endometrioid) FIGO grade (1 vs. 2 vs. 3) Myometrial invasion (no vs. < 50% vs. ≥ 50%) Primary tumor diameter (≤ 3 cm vs. > 3 cm)

ESMO: European Society of Medical Oncology; FIGO: International Federation of Gynecology and Obstetrics; GOG: Gynecologic Oncology Group; LVSI: lymphovascular space invasion; PORTEC: Post Operative Radiation Therapy in Endometrial Carcinoma; SEPAL: Survival Effect of Para-Aortic Lymphadenectomy in Endometrial Cancer

Table 5. Published preoperative risk prediction models for lymph node metastases

	Year	N =	Cohort	Histotype	Staging	Imaging	Predictors
Todo[145]	2003	214	Retrospective Multicenter Treatment between 1993—2000	Endometrioid and non-endometrioid	FIGO 1988 Stage I—IV	MRI	*Pelvic lymph node metastases:* Serous histotype (yes vs. no) Volume index (< 25 vs. ≥ 25) Grade (1—2 vs. 3) CA 125 (< 70 vs. ≥ 70 if age <50 years and < 28 vs. ≥ 28 if age ≥ 50 years) *Para-aortic lymph node metastases* Volume index (< 40 vs. ≥ 40) CA 125 (< 70 vs. ≥ 70 if age < 50 years and < 28 vs. ≥ 28 if age ≥ 50 years)
Lee[146]	2010	110	Retrospective Presumed one center Treatment between 2001—2008	Endometrioid and non-endometrioid	FIGO 1988 Stage I—IV	MRI	CA 125 (< 70 vs. ≥ 70) Any MI (yes/no) Grade (1 vs. 2—3) Extension beyond uterus (yes vs. no)
Kang[147]	2012	360	Retrospective Multicenter Treatment between 2002—2008	Endometrioid	FIGO 1988 Stage I—IV	MRI	CA 125 (< 35 vs. ≥ 35) MI (< 50% vs. ≥ 50%) Extension beyond corpus (yes vs. no) Enlarged lymph nodes (yes vs. no)
Koskas[148*]	2014	181	Retrospective Multicenter Treatment between 2000—2010	Endometrioid and non-endometrioid	Presumed FIGO 2009 Tentative stage I—II	MRI	Age (years) Race (white vs. black vs. others) Subtype (adenocarcinoma vs. papillary serous vs. clear cell vs. carcinosarcoma) Grade (1 vs. 2 vs. 3) Spread (no MI vs. MI < 50% vs. MI ≥ 50% vs. CSI)
Son[149]	2015	142	Retrospective One center Treatment between 2000—2013	Endometrioid grade 1—2	Presumed FIGO 2009 Tentative Stage IA	MRI	Grade 1 and CA 125 < 35 vs. grade 2 or CA 125 ≥ 35
Lee[25]	2016	172	Retrospective Presumed one center Treatment between 2000—2013	Endometrioid and non-endometrioid	FIGO 2009 Stage I—IV	TVS	MI (< 50% vs. ≥ 50%) Grade (1 vs. 2—3) CA 125 (< 35 vs. ≥ 35)

CSI: cervical stromal invasion; MI: myometrial invasion; MRI: magnetic resonance imaging; TVS: transvaginal ultrasound

** The nomogram by Bendifallah et al[14] used on preoperative variables*

1.8 SUMMARY

There is a need for improved preoperative risk assessment, to avoid under- or over treatment in terms of lymphadenectomy. Gynecologists use ultrasound in their everyday work. If they could assess deep myometrial invasion and cervical stromal invasion as good as ultrasound experts, that would aid in the triage of high-risk endometrial cancer, for referral to tertiary centers for surgery with lymphadenectomy.

The MELF pattern could constitute an adverse factor in endometrioid endometrial cancer and could be a reason for underestimating myometrial invasion, not only in histopathology but also in preoperative ultrasound staging.

Published preoperative risk prediction models for lymph node metastases are based on relatively small, retrospective cohorts, and the majority use MRI for imaging. The IETA 4 cohort[66] would enable model building on a large, multicenter, prospective cohort with high quality ultrasound data.

ProMisE poses the advantage over histotype and grade of higher agreement between biopsy and hysterectomy specimen[133, 135, 136], making it suitable for preoperative risk prediction. Possibly could ProMisE, demographic- and sonographic variables predict recurrence or progression already before surgery.

2 AIMS OF THE THESIS

The overall aim of this thesis was to improve preoperative risk assessment in women with endometrial cancer.

The specific aims were:

Study I

To assess interobserver reproducibility among ultrasound experts and gynecologists in the prediction of deep myometrial invasion and cervical stromal invasion by transvaginal ultrasound.

Study II

To describe sonographic features of the microcystic elongated and fragmented (MELF) pattern of myometrial invasion, to assess the effect of the MELF pattern on evaluation of myometrial invasion and to explore the relationship of the MELF pattern to more advanced stage (≥IB) and lymph node metastases.

Study III

To develop a preoperative risk prediction model on a large prospective cohort with variables from demography, endometrial biopsy and ultrasound, to estimate the individual risk of lymph node metastases.

Study IV

To assess demographic, sonographic and Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) variables, and their ability to predict recurrence or progression before surgery.

3 PARTICIPANTS AND METHODS

All study cohorts originate from the prospective, international, multicenter IETA 4 study[66] on women with endometrial cancer, examined preoperatively with ultrasound according to the IETA study protocol[40] (Figure 12).

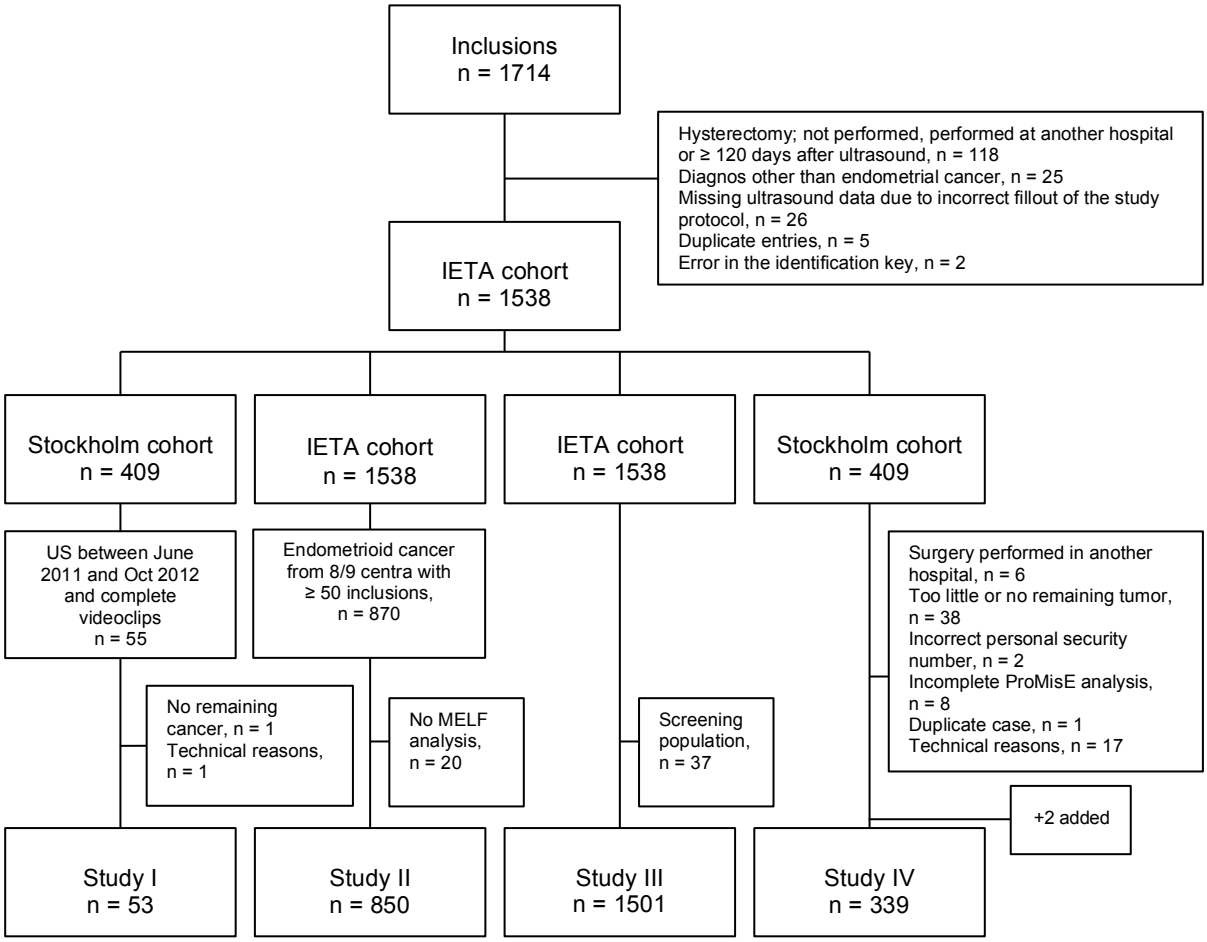

Figure 12. *Flowchart of the study populations.*

Women were recruited to the IETA 4 study between 1 January 2011 and 31 December 2015, from 17 European centers in 7 countries. Inclusion criteria were endometrial cancer according to preoperative biopsy or suspicion of cancer according to ultrasound, in the case of missing biopsy or biopsy without confirmed cancer (i.e. hyperplasia). Exclusion criteria were hysterectomy; not performed, carried out at another hospital or performed later than 120 days after ultrasound examination, final diagnosis other than endometrial cancer, incomplete data on ultrasound, duplicate entries and error in the identification key.

Ultrasound data was obtained by experienced ultrasound examiners, using high-end ultrasound equipment and following the IETA examination technique and terminology, previously described[40]. The IETA protocol included subjective assessment of the presence of deep myometrial invasion and cervical stromal invasion, measurements of maximum endometrial thickness and uterine AP diameter (sagittal section), uterine width (transverse

section), three maximum orthogonal tumor diameters; AP diameter and length (sagittal plane) and width (transverse plane), minimal tumor-free margin (in the plane where the distance from the tumor to the serosa appeared to be smallest), distance from the lower margin of the tumor to the outer cervical os (sagittal plane) and tumor volume (calculated from the three orthogonal tumor diameters using the approximate formula for an ellipsoid ((AP-diameter x length x width) /2) (Figure 1).

All women were subject to hysterectomy and bilateral salpingo-oophorectomy, with or without lymphadenectomy depending on local protocols. Tumors were staged according to the FIGO 2009 staging criteria[31] and grade was classified according to FIGO[79].

Data on demography and ultrasound variables were entered into an internet-based data capture software, Clinical Data Miner (https://cdm.esat.kuleuven.be)[153] before surgery and data on histopathology and surgical stage were entered following surgery. Incomplete forms could not be saved, which assured completeness of records. Once saved, data was locked to changes. The demographic data of the study cohorts is presented in Table 6.

Table 6. Demographic data on the study populations

	Study 1	Study 2	Study 3	Study 4
Number of women	53	850	1501	339
Age (years)	72 (62—77)	65 (58—72)	65 (59—72)	67 (60—72)
Body Mass Index (kg/m^2)	28 (24—34)	29 (24—34)	28 (24—33)	27 (24—33)
Histotype and grade*				
Endometrioid grade 1	16 (30)	295 (35)	572 (38)	141 (42)
Endometrioid grade 2	23 (43)	408 (48)	490 (33)	103 (30)
Endometrioid grade 3	9 (17)	147 (17)	210 (14)	46 (14)
Non-endometrioid	5 (9)	—	198 (13)	49 (14)
No remaining tumor	—	—	31 (2)	—
Stage FIGO 2009				
IA	34 (64)	527 (62)	904 (60)	205 (60)
IB	9 (17)	192 (23)	321 (21)	72 (21)
II	6 (11)	51 (6)	86 (6)	28 (8)
III	3 (6)	75 (9)	168 (11)	24 (7)
IV	1 (2)	5 (0.6)	22 (1.5)	10 (3)
Deep myometrial invasion	19 (36)	286 (34)	565 (38)	113 (33)
Cervical stromal invasion	9 (17)	76 (9)	158 (11)	41 (12)

Results are presented as median (IQR) or n (%)
**hysterectomy specimen*

3.1 PARTICIPANTS

3.1.1 Study I

The study cohort consisted of 53 women from the Stockholm (Sweden) center with endometrial cancer and complete videoclips of the uterine corpus and cervix, subject to preoperative ultrasound examination between June 2011 and October 2012.

3.1.2 Study II

The study cohort consisted of 850 women with endometrioid endometrial cancer from eight of the nine centers with 50 inclusions or more, subject to preoperative ultrasound examination between February 2011 and December 2015. The study cohort originated from five European countries (Czech Republic, Italy, Lithuania, Spain and Sweden).

3.1.3 Study III

The study cohort consisted of 1501 women with endometrial cancer, excluding one center recruiting women (n = 37) from a screening population, subject to preoperative ultrasound examination between January 2011 and December 2015. The study cohort originated from 16 centers in seven European countries (Belgium, Czech Republic, Hungary, Italy, Lithuania, Spain, Sweden).

3.1.4 Study IV

The study cohort consisted of 339 women from the Stockholm center (Sweden) with complete ProMisE analysis, subject to preoperative ultrasound examination between February 2011 and December 2015.

3.2 METHODS

3.2.1 Study I

To enable the assessment of an identical material of multiple cases by multiple users, we created a digitalized survey containing videoclips of the uterine corpus and the cervix in sagittal section, obtained by the same ultrasound expert prior to surgery. Through the survey, nine ultrasound experts, working as second-opinion sonographers around Europe, and nine Stockholm based gynecologists, using ultrasound in their everyday work but having no experience in ultrasound cancer staging, independently and subjectively evaluated myometrial invasion (< 50% / ≥ 50%), cervical stromal invasion (no / yes), videoclip quality (visual analogue scale 0—100), and certainty in the assessment (visual analogue scale 0—100). Each case needed to be finished to proceed to the next, but answers could be changed until the survey was submitted. Agreement to histopathology and interobserver reproducibility was measured and compared between the ultrasound experts and the gynecologists.

3.2.2 Study II

Reference pathologists, specialized in gynecological cancer, assessed the presence or the absence of the MELF pattern by re-evaluating the pathology slides from the hysterectomy specimen. Reference images and written information on the criteria of MELF diagnostics were sent to all pathologists, so as to ensure uniformity in assessment between centers. All evaluations in one center were done by the same pathologist, so as to ensure a uniform assessment within each center. The MELF pattern was confirmed when one of the three diagnostic criteria was met; 1) microcystic glands, 2) elongated glands or 3) fragmented small solid clusters or single cells. Sonographic features and accuracy of ultrasound assessment of myometrial invasion were compared in cases with the presence and the absence of the MELF pattern. The association of the MELF pattern to more advanced stage (≥IB) and lymph node metastases was assessed using univariable and multivariable logistic regression.

3.2.3 Study III

A mixed effects logistic regression model was developed, to calculate the individual risk of lymph node metastases. Candidate predictors were selected a priori, to avoid overfitting and to create a robust model[154]. Predictors that were highly subjective or subject to systematic measurement errors were not included. The number of predictors was chosen to yield 10 events per predictor, to guard against estimating too many parameters relative to the sample size[155]. The chosen predictors were age (years), duration of bleeding (months), results of preoperative biopsy (histotype and grade), tumor extension according to ultrasound (depth of myometrial invasion, cervical stromal invasion, extrauterine spread), endometrial color content (color score), tumor size (AP tumor/uterine diameter ratio) and "undefined tumor with unmeassurable endometrium", for tumors that could not be defined on ultrasound and where the endometrium could not be measured.

Missing data was multiply imputed, including lymph node metastases outcomes[156] in women without lymphadenectomy, assuming missingness not at random and missingness at random. Results obtained using imputed outcomes were compared through sensitivity analysis to results obtained from complete case analysis. The multivariable fractional polynomial algorithm was used to check for nonlinear effects of continuous predictors and simultaneously check if any of the a priori chosen predictors were redundant in the multivariable model[157]. The multicenter nature of the data was accounted for by forcing a center effect into the multivariable fractional polynomial algorithm. To avoid statistical overfitting, regression coefficients were shrunk using a heuristic shrinkage factor[158]. Exclusion of predictors was based on $\alpha = 0.2$.

The performance of the model was evaluated using leave-center-out cross validation[159]. Model performance was assessed by the discriminative ability to distinguish women with or without lymph node metastases (the area under the receiver operating characteristic (ROC) curve and sensitivity and specificity at various risk thresholds), by calibration to evaluate the reliability of the calculated risks (intercept, slope and calibration plot) and by clinical utility for decision making (decision curve analysis). All measures of model performance in each center were combined into global performance estimates using random-effects meta-analysis[160, 161]. The predictive performance of the model was compared with risk classification from endometrial biopsy alone (high risk: endometrioid cancer grade 3 or non-endometrioid cancer) and with combined endometrial biopsy and ultrasound (high risk: endometrioid cancer grade 3, non-endometrioid cancer, deep myometrial invasion, cervical stromal invasion or extrauterine spread).

3.2.4 Study IV

The ProMisE subtypes were analyzed retrospectively on formalin-fixed paraffin-embedded tumor tissue from a biobank, using immunohistochemistry (MMR-D, p53 wt and p53 abn) and gene mutation analysis by DNA sequencing (POLE EDM). Two pathologists reviewed all immunohistochemistry stains independently and resolved any interpretative discrepancies at a multiheaded microscope. ProMisE classification of the tumors was performed using the pragmatic model by Talhouk et al[132] (Figure 13). For practical reasons, ProMisE was analyzed on tumor tissue from the hysterectomy specimen, but was used as a proxy for the preoperative biopsy specimen throughout the study, as agreement between ProMisE on diagnostic biopsy and hysterectomy is high[133, 135, 136].

Data on adjuvant treatment, tumor recurrence, tumor progression and survival was obtained through review of digital patient files. All women were followed until death, loss of follow-up or end of follow-up (31 August 2019). Disease-free survival time was defined as time from surgery to detection of recurrence or end of follow-up and overall survival time as time from surgery until death of any cause or end of follow-up. The women were classified according to the pre- and postoperative ESMO classification[27] (Table 7).

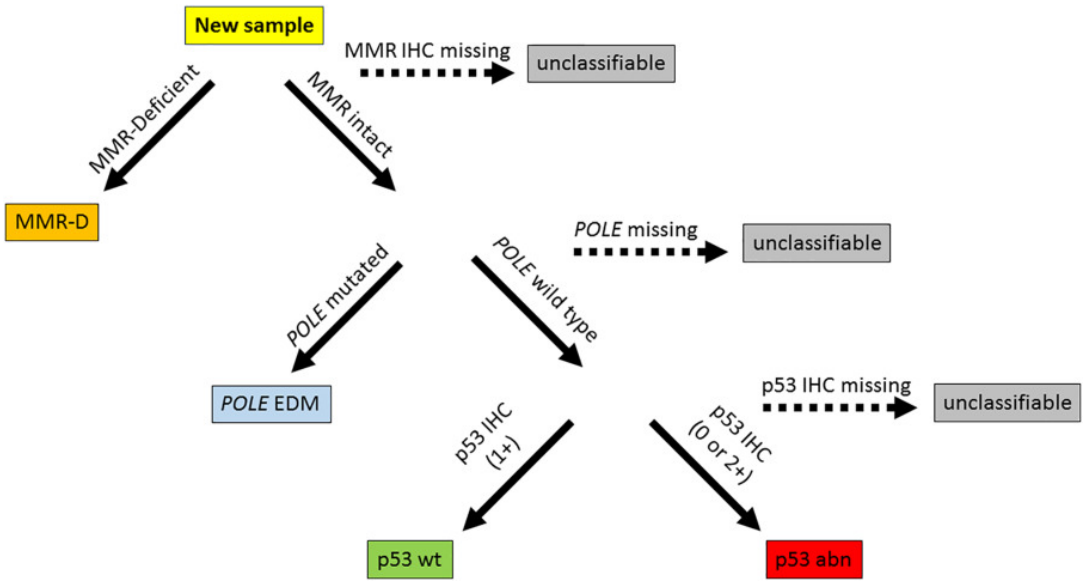

Figure 13. *Steps in molecular classification with Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE). Reprinted with permission*[132]
MMR-D: mismatch repair proteins deficiency; POLE EDM: polymerase-ε exonuclease domain mutations; p53wt: protein 53 wild type; p53 abn: protein 53 abnormal.

Cases with cervical stromal invasion and/or extrauterine spread according to ultrasound were added to the preoperative ESMO high-risk group. Due to the low number of women, the postoperative advanced and metastatic ESMO risk groups were combined.

Tumors with different ProMisE subtypes (MMR-D, POLE EDM, p53wt and p53 abn) were compared with regard to demographic-, sonographic- and histopathologic characteristics and to survival data, with special focus on the p53 abn subtype, as it is known to be associated with adverse outcome[120, 130-132, 136]. Variables associated with recurrence or progression were identified through univariable and multivariable Cox regression analysis.

To study if variables associated with progression or recurrence according to multivariable Cox regression analysis also had predictive value, they were analyzed using logistic regression models with a fixed time (outcome within three years). The predictive ability of the logistic regression models (ProMisE model and histotype and grade model) was compared to the logistic regression models for pre- and postoperative ESMO classification (ESMO pre and ESMO post), using area under the receiver operating characteristic (ROC) curves (AUC).

Table 7. Risk stratification according to the 2016 ESMO-ESGO-ESTRO Consensus statement Conference on Endometrial Cancer[27]

Type	Risk group	Description*
Preoperative†	Low	Endometrioid grade 1—2 with MI < 50%
	Intermediate	Endometrioid grade 1—2 with MI ≥ 50%
		Endometrioid grade 3 with MI < 50%
	High	Endometrioid grade 3 with MI ≥ 50%
		Non-endometrioid
Postoperative‡	Low	Stage I endometrioid, grade 1—2, < 50% MI, LVSI neg
	Intermediate	Stage I endometrioid, grade 1—2, ≥ 50% MI, LVSI neg
	High intermediate	Stage I endometrioid, grade 3, < 50% MI, LVSI neg or pos
		Stage I, endometrioid grade 1—2, LVSI pos, MI < or ≥ 50%
	High	Stage I endometrioid, grade 3, ≥ 50% MI, LVSI neg or pos
		Stage II
		Stage III without residual disease
		Non-endometrioid
	Advanced	Stage III with residual disease
		Stage IVA
	Metastatic	Stage IVB

**FIGO 2009 staging is used*
†To guide decision on lymphadenectomy
‡To guide adjuvant therapy use
LVSI: lymphovascular space invasion; MI: myometrial invasion

3.3 STATISTICAL ANALYSIS

The chi-square test (**Study I**) and Fisher's exact test (**Study IV**) were used for categorical data. The t-test was used for normally distributed continuous data (**Study I**), the Mann-Whitney U test for non-normally distributed continuous data comparing two groups (**Study I, II and IV**) and the Kruskal-Wallis test for continuous data comparing more than two groups (**Study IV**). McNemars test was used for paired categorical data (**Study I**). Kappa statistics was used for agreement, with Cohen's kappa for a single observer and Fleiss Kappa for multiple observers (**Study I**). Spearman's correlation coefficient was used to assess statistical dependence between two variables (**Study I**). Logistic regression analysis (**Study II—IV**) and Cox regression analysis (**Study IV**) were used to study crude (univariable analysis) and adjusted (multivariable analysis) associations between variables and outcomes. Multiple imputation was used to impute missing data (**Study III**). The multivariable fractional polynomial algorithm was used to develop a mixed effects logistic regression model (**Study III**). Leave-center-out cross validation was used to evaluate model performance (**Study III**). Discriminative ability of logistic regression models was assessed through AUC (**Study III and IV**) and sensitivity and specificity at various risk thresholds (**Study III**). Pairwise comparison of AUC was performed using DeLong test (**Study IV**). Diagnostic performance was assessed through sensitivity, specificity (**Study I and II**), positive predictive value (PPV), negative predictive value (NPV) and accuracy (**Study II**). Decision curve analysis was used to assess clinical utility (**Study III**). Survival analysis (disease-free survival and overall survival) was estimated from Kaplan-Meier plots, with the log rank test for testing differences in survival (**Study IV**). P-value < 0.05 was considered statistically significant (**Study I—IV**).

An introduction to used statistical methods is given below, in order of appearance in the thesis.

3.3.1 Diagnostic accuracy

Sensitivity is the ability of a test to correctly identify those with the condition (true positive rate) whereas specificity is the ability of a test to correctly identify those without the condition (true negative rate). False positive rate (FPR) = 1 - specificity and false negative rate (FNR) = 1 - sensitivity. A perfect test would have 100 % sensitivity, i.e. identifying all with the condition, and 100 % specificity, i.e. not identifying anyone without the condition as having it. Sensitivity and specificity are independent of the percentage of positive cases in the population of interest (i.e. the prevalence), in comparison to the predictive values. Positive predictive value (PPV) refers to the test making a positive prediction and the subject having a positive result and the negative predictive value (NPV) refers to the test making a negative prediction and the subject having a negative result. Accuracy corresponds to the proportion of correctly classified cases[162] (Figure 14).

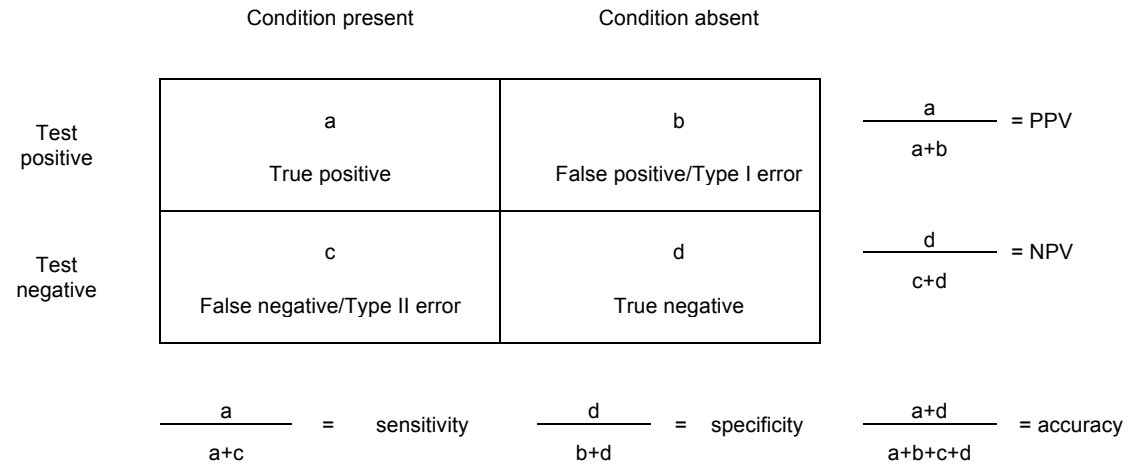

Figure 14. *Two-by-two contingency table for diagnostic tests*
NPV; negative predictive value; PPV: positive predictive value

3.3.2 Interobserver reproducibility

The kappa (κ) statistics provides a quantitative measure of the magnitude of agreement between observers in the interpretation of a specific test and can be measured when two or more independent observers evaluate the same material. When measuring interobserver reproducibility, one must take into account that observers might agree or disagree simply by chance.

The kappa statistic is based on the difference between "observed agreement" (how much agreement is actually present) and “expected agreement” (how much agreement that would be expected to be present by chance alone). The result is given on a scale of -1 to +1, where 1 is perfect agreement, 0 is agreement equivalent to chance and < 0 is agreement less than chance (systematic disagreement between observers)[163, 164].

Kappa values were categorized as "poor" (≤ 0.20), "fair" (0.21—0.40), "moderate" (0.41—0.60), "good" (0.61—0.80) and "very good) (0.81—1.00)[165].

3.3.3 Logistic regression

Logistic regression is used to predict a binary outcome (1/0, yes/no, true/false) given one or more independent variables. It predicts the probability of an event by fitting data to a logit function (log odds). The result is the impact of each variable on the odds ratio (OR) of the outcome.

Odds are the ratio between probabilities, i.e. between the probability of an event favorable to an outcome and the probability of an event against the same outcome. The odds ratio is the ratio between odds.

A large odds ratio means that the risk of a particular group is much greater than that of the reference group, and may or may not be indicative of a large probability of an outcome. If the risk of the reference group is small, even a large odds ratio can indicate a small probability[166].

3.3.4 Multiple imputation

As there is usually a reason for missing values, exclusion of cases with missing data with analysis only on complete cases can lead to a biased sample and poor generalizability. Furthermore, it reduces the sample size, with subsequent loss of precision and power. The risk of missing data causing bias depends on the reasons why data are missing. Commonly, reasons for missing data are classified as; missing completely at random, MCAR (no systematic differences between missing and observed values), missing at random, MAR (any systematic differences between missing and observed values can be explained by differences in observed data) and missing not at random, MNAR (remaining systematic differences between the missing and the observed values, even after the observed data are taking into account).

Multiple imputation analysis is a statistical method to deal with missing data. It avoids bias induced by systematic differences by allowing also individuals with incomplete data to be included in analyses and enables all patient data to be used in the building of a risk prediction model. Multiple imputation is especially useful when auxiliary variables, such as hysterectomy findings, are available to impute the outcome[156].

In multiple imputation, multiple datasets are created, with replacement of the missing values based on their predictive distribution in the observed data. The risk prediction model is fitted to each of the imputed datasets and estimated associations are averaged together to give overall estimated associations. Bias is avoided only if enough variables predictive of the missing values are included in the imputation model[167, 168].

3.3.5 Leave-center-out cross validation

If a model is tested on the same multicenter dataset on which it was developed, a cross-validation procedure can be used that mimics external validation. The model is repeatedly developed in all centers but one and validated in the remaining center. If the models perform well in leave-center-out cross validation, a model derived from the entire multicenter dataset is likely to be generalizable[159].

3.3.6 Receiver Operating Characteristic (ROC) curves

A ROC-curve is a graphical plot that illustrates the performance of a binary classifier system at various discrimination thresholds between 0 and 1. It is created by plotting the true positive rate (sensitivity) against the false positive rate (1-specificity) and illustrates how a test or a predictive model can discriminate between true positives and true negatives. The diagonal reference line represents the ROC curve for a test or model without discriminative ability. The further in the upper left the curve of the test or model is from the diagonal line, the better the discrimination. The area under the ROC curve (AUC) is the most commonly used ROC index. An AUC of 1 reflects a test or model with perfect discrimination whereas an AUC of 0.5 reflects absent discriminative ability[169].

3.3.7 Calibration analysis

The calibration plot is a graphical plot of the agreement between predicted and observed outcomes. A model that is well calibrated will yield a curve lying on the diagonal, suggesting that predicted risks are correct, i.e. among subjects with a predicted risk of 15%, 15 out of 10 will have the event.

Ideally, the calibration slope is one and the calibration intercept is zero. A calibration slope deviating from one indicates over- or underfitting. Overfitting (slope < 1) yields predicted risks in high-risk women that are too high, and predicted risks in low-risk women that are too low, while underfitting (slope > 1) yields predicted risks that are too moderate, i.e. high risks are underestimated while low risks are overestimated.

A calibration intercept deviating from zero indicates miscalibration-in-the-large: on average in this population, the risk is overestimated (< 0) or underestimated (> 0). Miscalibration-in-the large can occur simultaneously with over- or underfitting[170].

3.3.8 Decision curve analysis

Decision curve analysis is a tool to quantify the clinical utility for decision making for a risk prediction model. It balances the benefits of true positives against the harms of false positives on a single scale, by using a weighting factor for false positives. The weighted factor corresponds to the odds of the chosen risk threshold for performing a certain procedure, such as biopsy or surgery.

A risk threshold of 10% corresponds to the odds 1:9, i.e. the benefit of performing the procedure in someone with the outcome is considered nine times as large as the harm of

performing the procedure in someone without the outcome. The decision curves (net benefit vs. risk thresholds) are plotted for the risk prediction model and compared to the default strategies of performing the procedure in all and in none. If one curve is highest over the full range of probability thresholds, then that diagnostic approach is the best[171, 172].

3.3.9 Cox regression

Cox regression analysis, also called proportional hazards regression, investigates the relationship of predictors and the time-to-event through a hazard function. It differs from logistic regression by assessing a rate (the number of new cases of an outcome per population at risk per unit of time, i.e. the incidence or hazard rate) instead of a proportion (the proportion of new cases that develop in a given time period, i.e. the cumulative incidence).

Unlike logistic regression, Cox regression is dependent on time. The Cox regression analysis estimates the hazard ratio (HR), which can be interpreted as the relative risk of the event occurring at time t, or the relative event rate. A HR > 1 means that the event is more likely to occur, and a HR < 1 that the event is less likely to occur. If the HR = 1, the predictor has no effect on the hazard of the event[173].

3.3.10 Kaplan-Meier analysis

The Kaplan-Meier curve is a graphical method for displaying survival or time-to-event analysis. The Kaplan-Meier curve shows what the probability of an event is at a certain time interval. The horizontal axis represents the time from enrollment, corresponding to the time at which participants are considered at-risk for the outcome of interest, and the vertical axis represents the estimated probability of survival, i.e. cumulative survival. Each downward step in the lines represents an event (the outcome of interest), while each small vertical tick represents a censored observation. Censoring means that the total survival time for that subject cannot be accurately determined. This occurs when the individual drops out of the study, is lost to follow-up or the study ends before the subject had the event of interest. With time, fewer people remain at risk. Survival curves can be compared using the HR or through the log rank test[174].

4 RESULTS

4.1 STUDY I

No significant difference could be found between ultrasound experts and gynecologists in the diagnostic accuracy and agreement with histopathology for deep myometrial invasion. However, ultrasound experts had significantly higher diagnostic accuracy and greater agreement to histopathology for cervical stromal invasion (Table 8). Ultrasound experts rated their certainty as being greater than did gynecologists, in the assessment of both deep myometrial invasion and cervical stromal invasion (Table 8). Certainty in assessment, for ultrasound experts and gynecologists combined, was correlated with correct assessment of both deep myometrial invasion and cervical stromal invasion (correlation coefficient 0.49, $p < 0.01$ vs. 0.62, $p < 0.01$, respectively). Ultrasound experts and gynecologists assessed videoclip quality similarly, regarding both deep myometrial invasion and cervical stromal invasion (Table 8). Videoclip quality, for ultrasound experts and gynecologists combined, did not affect the correct assessment of deep myometrial invasion, but was correlated to correct assessment of cervical stromal invasion (correlation coefficient 0.24, $p = 0.08$ vs. 0.36, $p = 0.01$). Neither BMI nor preoperative risk (low risk: endometrioid cancer grade 1—2 and high risk: endometrioid cancer grade 3 or non-endometrioid cancer) did affect correct assessment of deep myometrial invasion or cervical stromal invasion (BMI: correlation coefficient 0.04, $p = 0.8$ vs. -0.11, $p = 0.4$, respectively; preoperative risk: correlation coefficient 0.74 vs. 0.63, $p = 0.4$ and 0.80 vs. 0.76, $p = 0.6$, respectively).

Table 8. Assessment of deep myometrial invasion (≥ 50%) and cervical stromal invasion by ultrasound experts and gynecologists

a. Deep myometrial invasion

	Ultrasound experts		Gynecologists		
	Value	95% CI	Value	95% CI	*p**
Sensitivity (%)	73	66 —79	73	66 —79	1.00
Specificity (%)	69	63 —74	70	65—75	0.68
Kappa†	0.52	0.48 —0.57	0.48	0.44 —0.53	0.11
Certainty (VAS)	74	63 —84	56	48 —65	< 0.01
Quality (VAS)	64	52 —77	56	50 —63	0.23

b. Cervical stromal invasion

	Ultrasound experts		Gynecologists		
	Value	95% CI	Value	95% CI	*p**
Sensitivity (%)	57	45 —68	42	31 —53	<0.01
Specificity (%)	87	83 —90	83	78 —86	0.02
Kappa†	0.58	0.53 —0.62	0.45	0.40 —0.49	<0.01
Certainty (VAS)	77	65 —89	58	48 —68	0.01
Quality (VAS)	71	57 —85	59	53 —64	0.07

Certainty: certainty in assessment, as measured on a visual analogue scale
Quality: experienced videoclip quality, as measured on a visual analogue scale
** Mc Nemar's test † Fleiss kappa for multiple observers*

Interobserver reproducibility for deep myometrial invasion did not differ between ultrasound experts and gynecologists. The test proportion "good" and "very good" was 34% vs. 22% (p = 0.13), respectively. Interobserver reproducibility for cervical stromal invasion was significantly higher for ultrasound experts compared to gynecologists, with a test proportion "good" and "very good" of 53% vs. 14% ($p < 0.01$) (Table 9).

Table 9. Interobserver reproducibility of ultrasound experts and gynecologists in the assessment of deep myometrial invasion (≥ 50 %) and cervical stromal invasion

	Deep myometrial invasion n (%)		Cervical stromal invasion n (%)	
Kappa	Ultrasound experts	Gynecologists	Ultrasound experts	Gynecologists
Poor (< 0.20)	0 (0)	0 (0)	0 (0)	3 (8)
Fair (0.21—0.40)	7 (19)	7 (19)	4 (11)	11 (31)
Moderate (0.41—0.60)	27 (47)	21 (58)	13 (36)	17 (47)
Good (0.61—0.80)	11 (31)	8 (22)	19 (53)	5 (14)
Very good (0.81—1.00)	1 (3)	0 (0)	0 (0)	0 (0)

4.2 STUDY II

The MELF pattern was found in 23% of the women, and was associated with older age (68 vs. 64 years, $p < 0.01$), lower body mass index (BMI) (29 vs. 30, $p < 0.01$), post-menopausal status (95% vs. 87%, $p < 0.01$), deep myometrial invasion (52% vs. 28%, $p < 0.01$), cervical stromal invasion (13% vs. 8%, p =0.04), lymph node metastases (26% vs. 14%, p = 0.01) and higher stage (stage ≥IB: 56% vs. 33%, $p < 0.01$) but not with high-grade (grade 3: 16% vs. 18%, p =0.51).

Tumors with the MELF pattern were two to three times more likely to have deep myometrial invasion (OR 2.8, 95% CI 2.0—3.9, $p < 0.01$), cervical stromal invasion (OR 1.7, 95% CI 1.0—2.9, p = 0.04), higher stage (stage ≥IB: OR 2.6, 95% CI 1.9—3.6, $p < 0.01$) and lymph node metastases (OR 2.2, 95% CI 1.2—4.0, p = 0.01) in univariable analysis.

On preoperative ultrasound, tumors with the MELF pattern were slightly larger (endometrial thickness 20 mm vs. 18 mm, p = 0.03), had a higher endometrial color score (score 3—4: 68% vs. 56%, $p < 0.01$) and more often the multiple multifocal vascular pattern (48% vs. 34%, $p < 0.01$), whereas little difference was seen in the assessment of endometrial morphology (non-uniform echogenicity: 68% vs. 62%, p = 0.11; non-regular endometrial-myometrial junction: 82% vs. 75%, p = 0.06; bright edge present: 9% vs. 13%, p = 0.11).

Ultrasound assessment of deep myometrial invasion was not affected by the MELF status, as there was no difference in sensitivity of specificity compared to tumors with and without the MELF pattern. The positive predictive value was higher and the negative predictive value lower in tumors with the MELF pattern (Table 10). Ultrasound underestimated myometrial invasion to the same degree in tumors with and without the MELF pattern (29.1% vs. 29.5%, p = 0.95).

Table 10. Sonographic assessment of deep myometrial invasion in relation to the MELF pattern (%)

	No MELF	MELF	*p**
Sensitivity	71	71	0.95
Specificity	79	76	0.41
Accuracy	77	73	0.28
PPV	57	76	< 0.01
NPV	87	70	< 0.01

** $\chi 2$ test*

PPV: positive predictive value; NPV negative predictive value

According to histopathology, MELF status and grade were significantly associated to higher stage (≥IB) in both univariable- and multivariable analysis. MELF, deep myometrial invasion and cervical stromal invasion, but not grade, were significantly associated with lymph node metastases in univariable analysis, whereas only deep myometrial invasion and cervical stromal invasion remained significantly associated to lymph node metastases in multivariable analysis (Table 11).

Table 11. Univariable- and multivariable logistic regression analysis modeling stage ≥ IB (n = 850) and lymph node metastases (n = 348)

a. Stage ≥ IB

	Univariable analysis			Multivariable analysis*		
	OR	95 % CI	*p*	OR	95 % CI	*p*
MELF present	2.6	1.9—3.6	< 0.01	2.3	1.6—3.2	< 0.01
Grade 1	Ref			Ref		
Grade 2	2.6	1.9—3.7	< 0.01	2.4	1.7—3.4	< 0.01
Grade 3	4.7	3.1—7.2	< 0.01	4.6	3.0—7.2	< 0.01
MI ≥ 50%	—			—		
CSI	—			—		
MI < 50%, no CSI	—			—		
MI ≥ 50%, no CSI	—			—		
CSI, MI <50% / ≥ 50%	—			—		

b. Lymph node metastases

	Univariable analysis			Multivariable analysis*		
	OR	95 % CI	*p*	OR	95 % CI	*p*
MELF present	2.2	1.2—4.0	0.01	1.8	0.9—3.5	0.09
Grade 1	Ref			Ref		
Grade 2	1.4	0.6—3.3	0.51	1.0	0.4—2.7	0.94
Grade 3	2.4	1.0—5.9	0.06	2.1	0.7—5.7	0.16
MI ≥ 50%	8.0	3.8—16.8	< 0.01	—		
CSI	4.0	2.1—7.6	< 0.01	—		
MI < 50%, no CSI	Ref			Ref		
MI ≥ 50%, no CSI	5.6	2.5—12.2	< 0.01	4.9	2.2—11.4	< 0.01
CSI, MI <50% / ≥ 50%	10.8	4.5—25.8	< 0.01	9.6	3.9—23.8	< 0.01

**Adjusted for age, body mass index and menopausal status*

CI: confidence interval; CSI: cervical stromal invasion; MELF: microcystic, elongated and fragmented; MI: myometrial invasion

4.3 STUDY III

Median age was 65 years and the majority of the women (87%) reported abnormal bleeding, with a median bleeding duration of three months. Most women had low-grade endometrioid cancer in the preoperative biopsy (69%) and tentative stage IA, according to preoperative ultrasound (60%). Of the 691 women (46%) subject to lymphadenectomy, lymph node metastases were present in 127 (18%). Of the 252 women (17%) with undefined tumor on ultrasound, the AP tumor diameter was replaced by endometrial thickness when measurable (15%), or was accounted for as a separate variable when not measurable (2%). Preoperative grade was missing in 75 women (5%) and lymph node status in 810 women (54%), and were multiply imputed.

The preoperative predictors are presented in Table 12. The color Doppler score did not meet the criterion for inclusion ($\alpha = 0.2$) and was not retained as a predictor. High-grade did not add to the risk of lymph node metastases, when adjusting for the other predictors.

Table 12. Adjusted associations between the preoperative predictors and the presence of lymph node metastases on the basis of logistic regression (n = 1501)

Predictor	OR	95 % CI
Intercept		
Age (per 10 years)	1.16	0.92—1.48
Bleeding duration (per 3 months)	1.09	0.99—1.20
Endometrial biopsy		
Endometrioid grade 1—2	Ref	
Endometrioid grade 3	0.92	0.52—1.65
Non-endometrioid	2.01	1.22—3.33
Other*	1.89	0.58—6.12
Ultrasound tumor extension		
MI < 50%, no CSI	Ref	
MI ≥50%, no CSI	1.75	1.00—3.07
CSI ± MI ≥ 50%	1.77	0.84—3.70
Extrauterine spread	5.86	2.98—11.88
Color Doppler†		
Score 1—2	Ref	
Score 3—4	1.20	0.68—2.10
Invisible endometrium	1.01	0.36—2.87
AP tumor/uterine diameter ratio (per 0.25)	1.49	1.13—1.97
Undefined tumor with unmeasurable endometrium	3.41	0.76—15.22

Exclusion of predictors was based on α = 0.2, hence 95 % CI including 1 is not sufficient for exclusion

** Biopsy with complex atypical hyperplasia or no biopsy, but ultrasound findings consistent with cancer*

† Not retained (p > 0.2)

AP: anteroposterior; CI: confidence interval; CSI; cervical stromal invasion; MI: myometrial invasion

The model formula is presented in Figure 15. The individual risk of lymph node metastases is obtained by multiplying each predictor by its regression coefficient and the addition of the intercept.

The overall AUC of the risk prediction model is 0.73 (95 CI 0.68—0.78). The model is well calibrated (calibration slope 1.06 and calibration intercept 0.06), meaning that predicted risks correspond well to observed lymph node metastases frequencies. The adjusted odds ratios (Table 12) indicate that the model predicts the risk of lymph node metastases better than the use of biopsy only or combined biopsy and ultrasound, as additional model inputs contribute to the prediction of lymph node metastases even if ultrasound tumor extension and biopsy results are taken into account. For example, a 3-month increase in bleeding duration increases the odds of lymph node metastases with 9% (adjusted OR 1.09, 95% CI 0.99—1.20).

Probability (lymph node metastases = yes) = $\frac{1}{1+exp(-X\beta)}$

Xβ =
-3.962
+ 0.134 × (age in years / 10)
+ 0.072 × (duration of abnormal bleeding in months / 3)
+ 0.607 × (1 if non-endometrioid cancer on endometrial biopsy, 0 otherwise)
+ 0.592 × (1 if clinical suspicion of cancer*, 0 otherwise)
+ 0.502 × (1 if MI ≥ 50% without CSI or extrauterine spread on ultrasound, 0 otherwise)
+ 0.512 × (1 if CSI without extrauterine spread on ultrasound, 0 otherwise)
+ 1.533 × (1 if extrauterine spread on ultrasound, 0 otherwise}
+ 0.359 × (AP tumor/uterine diameter ratio / 0.25)
+1.046 × (1 if undefined tumor with unmeasurable endometrium, 0 otherwise)

Figure 15. *Model formula with regression coefficients obtained by heuristic shrinkage*
**Endometrial biopsy with other diagnosis, e.g. complex atypical hyperplasia, or no biopsy, but ultrasound findings consistent with cancer*
AP: anteroposterior; CSI: cervical stromal invasion; MI: myometrial invasion

Whereas risk stratification by biopsy only or combined biopsy and ultrasound have fixed sensitivity and specificity levels, the model threshold can be shifted upward, for a better specificity, or downward for a better sensitivity (Table 13). The lower the risk threshold, the more lymph node metastases are correctly identified, but at the cost of a higher number of unnecessary lymphadenectomies. When setting the model threshold to give equally large test positive groups as biopsy only and combined biopsy and ultrasound, the risk prediction model proved superior, as it was associated with higher sensitivity without any loss of specificity (Table 13).

Table 13. Model performance depending on risk threshold in distinguishing between women with and without lymph node metastases and comparison between the risk prediction model and risk-classification by preoperative biopsy alone or combined biopsy and ultrasound (n = 1501)

Model risk threshold	Test positive	95 % CI	Sensitivity	95 % CI	Specificity	95 % CI
0.03	97	96—98	99	94—100	3	2—4
0.05	90	88—92	98	92—99	11	9—14
0.1	58	54—62	83	76—89	47	41—52
0.15	39	35—43	67	58—75	66	61—71
0.2	24	21—28	48	39—56	80	75—85
0.3	9	7—12	25	18—33	94	90—96
0.4	4	3—6	13	8—20	97	95—99
0.5	2	1—3	5	3—11	99	97—100
Comparison of the model, biopsy alone and combined biopsy and ultrasound						
0.196*	25	22—29	50	41—58	80	74—84
Biopsy†	25	21—29	35	27—44	77	72—82
0.113*	52	48—56	80	71—86	53	47—58
Biopsy + ultrasound‡	52	47—57	75	66—81	52	46—59

Results are from leave-center-out cross validation
**Model thresholds chosen to give equally large test positive groups as "Biopsy" and "Biopsy and ultrasound"*
† Endometrial biopsy (endometrioid grade 3/non-endometrioid)
‡ Endometrial biopsy (endometrioid grade 3/non-endometrioid) and ultrasound extension (deep myometrial invasion/cervical stromal invasion/extrauterine spread)

The clinical utility of the competing strategies were compared using decision curve analysis, together with the default strategies of conducting lymphadenectomy in all and in none (Figure 16). The model proved superior to biopsy only and combined biopsy and ultrasound, as the net benefit curve of the model was higher over all risk thresholds, indicating better outcomes when the harms of false positives (unnecessary lymphadenectomies) and false negatives (missed lymph node metastases) are taken into account.

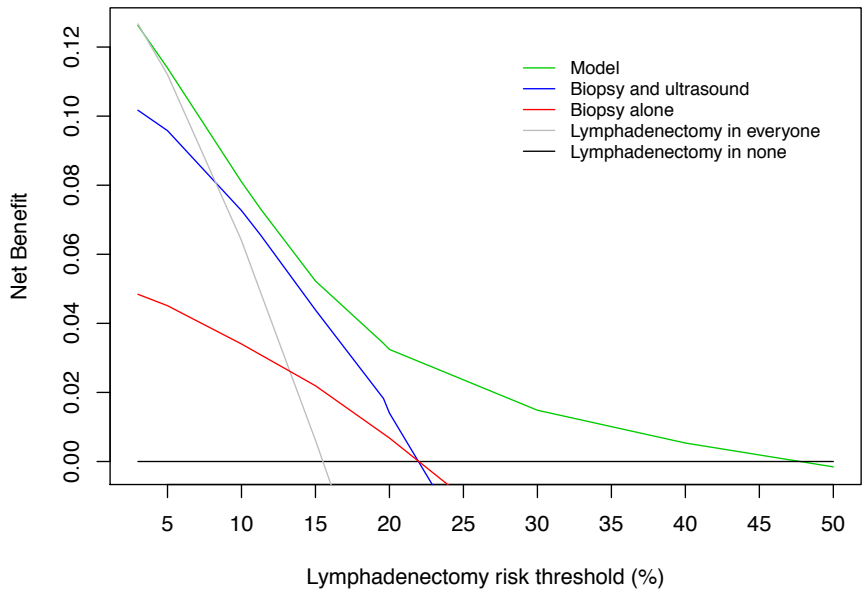

***Figure 16**. Decision curves representing the net benefit of the risk model, combined biopsy and ultrasound, biopsy alone, lymphadenectomy in everyone and lymphadenectomy in none for risk thresholds between 3% and 50%. Results are from leave-center-out cross validation.*

4.4 STUDY IV

Median follow-up time from surgery was 58 months. Recurrence or progression occurred in 15% of all women (14% of MMR-D, 8% of POLE, 9% of p53 wt and 46% of p53 abn). Compared to those without, the 51 women with recurrence or progression were older (70 vs. 66 years, p <0.01), had larger waist-circumference (105 cm vs. 93 cm, p = 0.02), more advanced tumor extension on ultrasound (deep myometrial invasion, cervical stromal invasion or extrauterine spread: 69% vs. 29%, p < 0.01), larger ultrasound tumor size (AP diameter ≥ 2 cm: 83% vs. 36%, p < 0.01), higher endometrial color score (score 3—4: 78% vs. 62%, p =0.04), higher tumor stage (stage ≥ IB: 80% vs. 32%, p = < 0.01), more non-endometrioid cancer (39% vs. 10%, p < 0.01) and the ProMisE p53 abn subtype (39% vs. 8%, p < 0.01). The vast majority of recurrences (88%) occurred within three years, and all tumor progressions occurred within two years.

Compared to the other ProMisE subtypes, women with p53 abn had a significantly higher probability of recurrence or progression (46% vs. 11%, p < 0.01) (Figure 17), were older (70 years vs. 67 years, p = 0.04), had larger ultrasound tumor size (AP diameter 26 mm vs. 17 mm, p < 0.01), more often non-endometrioid cancer (77% vs. 5%, p < 0.01), higher stage (stage ≥II: 41% vs. 15%, p <0.01), decreased 5-year disease free survival (51% vs. 86%, p < 0.01) and more often death from disease (39% vs. 5%, p < 0.01).

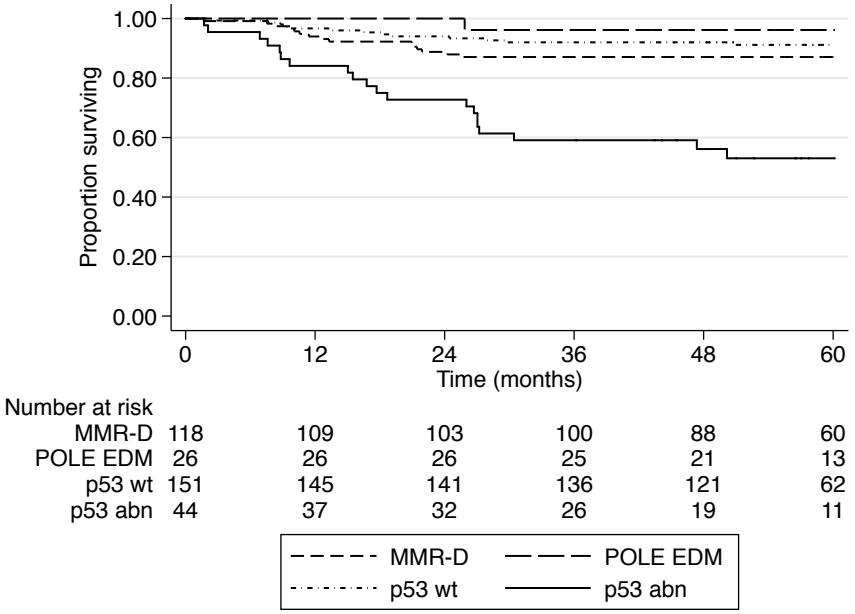

Figure 17*. Kaplan-Meier plot on recurrence or progression for the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) subtypes (n = 339)*
Overall p < 0.01
MMR-D vs. p53 abn: p < 0.01
POLE EDM vs. p53 abn: p < 0.01
p53 wt vs. p53 abn: p < 0.01

Multivariable Cox regression analysis, with all significant preoperative variables from univariable analysis, revealed that age ≥ 65 years, waist circumference ≥ 88 cm, ProMisE, ultrasound tumor extension and ultrasound tumor AP diameter ≥ 2 cm were independently associated with recurrence or progression (Table 14), an association that remained also when we adjusted for the postoperative ESMO classification. When ProMisE was removed from the multivariable analysis, histotype and grade also became significantly associated with the outcome, indicating an interaction between these two variables. Hence, ProMisE and histotype and grade were accounted for in separate models.

Table 14. Multivariable Cox regression analysis; associations of preoperative variables on tumor recurrence or progression (n=339)

	All variables significant in univariable analysis			Histotype and grade model			ProMisE model		
	HR	95 % CI	p^*	HR	95 % CI	p^*	HR	95 % CI	p^*
Demographic variables									
Age (years)			< 0.01			< 0.01			< 0.01
< 65	Ref			Ref			Ref		
≥ 65	4.0	1.7—9.5		4.4	2.0—9.8		3.8	1.7—8.4	
Waist circumference (cm)			0.01			0.01			0.02
< 88	Ref			Ref			Ref		
≥ 88	2.6	1.2—5.6		2.5	1.2—5.1		2.5	1.2—5.1	
Histopathological variables									
Histotype and grade			0.40			< 0.01			
Endometrioid grade 1—2	Ref			Ref			—	—	
Endometrioid grade 3	2.0	0.8—5.0		2.6	1.1—6.0		—	—	
Non-endometrioid	1.9	0.7—4.8		4.4	2.3—8.2		—	—	
Other†	0.8	0.2—3.5		0.8	0.2—3.4		—	—	
ProMisE			0.04						< 0.01
p53 wt	Ref			—	—		Ref		
MMR-D	1.1	0.5—2.4		—	—		1.1	0.5—2.4	
POLE EDM	1.0	0.2—5.1		—	—		1.3	0.3—6.3	
p53 abn	3.9	1.3—11.1		—	—		5.7	2.8—11.7	
Ultrasound variables									
Tumor extension			< 0.01			< 0.01			< 0.01
MI < 50%, no CSI	Ref			Ref			Ref		
MI ≥ 50%, no CSI	1.4	0.5—3.5		1.4	0.6—3.0		1.6	0.7—3.5	
CSI present ± MI ≥ 50 %	1.8	0.6—5.5		2.2	0.9—5.3		2.2	0.9—5.4	
Extrauterine spread	9.7	3.0—30.7		7.4	2.8—19.7		11.	4.2—31.0	
Tumor AP diameter (cm)			< 0.01			< 0.01			0.01
<2	Ref			Ref			Ref		
≥ 2	4.7	1.8—12.4		3.9	1.6—9.7		3.8	1.6—9.4	
Tumor not defined	5.3	1.02—27.2		4.2	1.1—16.7		3.8	0.96—15.3	
Endometrial-myometrial junction‡			0.30						
Regular	Ref			—			—	—	
Irregular/interrupted/undef	2.0	0.6—7.4		—			—	—	
Endometrial morphology‡			0.30						
Uniform	Ref			—			—	—	
Non-uniform	1.3	0.7—2.4		—			—	—	
Color score‡			0.07						
1—2	Ref			—			—	—	
3—4	0.4	0.2—1.1		—			—	—	
Vascular pattern‡			0.60						
Other	Ref			—			—	—	
Multiple multifocal	1.2	0.5—3.0		—			—	—	

** Test of variable including all categories*

† Endometrioid cancer not graded (n=5), suspicion of endometrial cancer (n=24), no biopsy (n=1)

‡ In the 332 cases with visible endometrium on ultrasound

AP: anteroposterior; CI: confidence interval; CSI: cervical stromal invasion; MI: myometrial invasion; undef: undefined

Ultrasound tumor size remained significantly associated to recurrence or progression in all versions of multivariable analysis. In women with defined tumor on ultrasound (n = 317), tumors with AP diameter ≥ 2 cm, as compared to < 2 cm, were associated with deep myometrial invasion (56% vs. 16%, $p < 0.01$), lymph node metastases (30% vs. 7%, $p = 0.03$), worse 5-year overall survival (78% vs. 93%, $p < 0.01$) and higher risk of recurrence or progression, also among the 154 women with preoperative ESMO low risk (15% vs. 3%, $p = 0.03$).

Stratification by tumor size (tumor AP diameter < 2 cm vs. ≥ 2 cm) and p53 abn status (non-p53 abn vs. p53 abn) identified a large group of women (48% of the study population) with a very low risk of recurrence or progression (1.8%) (Figure 18).

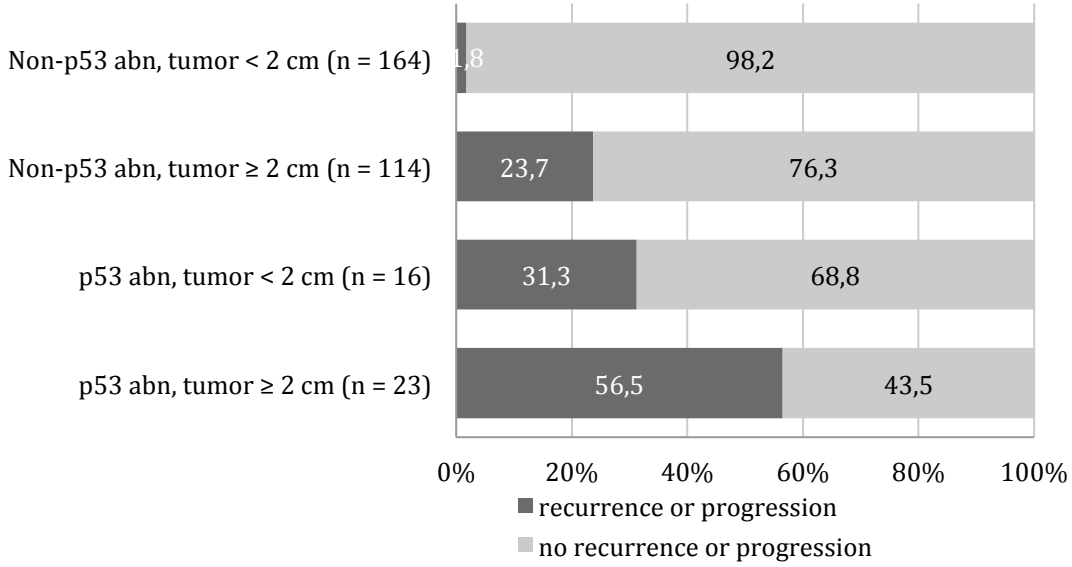

Figure 18. *Risk of recurrence or progression in relationship to p53 abn status and ultrasound tumor size (n = 317).*
Non-p53 abn: MMR-D, POLE EDM or p53 wt.
Tumor < 2 cm vs. ≥ 2 cm: anteroposterior tumor diameter < 2 cm vs. ≥ 2 cm

The ProMisE model predicted recurrence or progression with comparable ability as the histotype and grade model (AUC 0.89 vs. 0.88, $p = 0.22$) and with higher ability than both preoperative ESMO classification (AUC 0.89 vs. 0.74, $p < 0.01$) and postoperative ESMO classification (AUC 0.89 vs. 0.79, $p < 0.01$) (Figure 19).

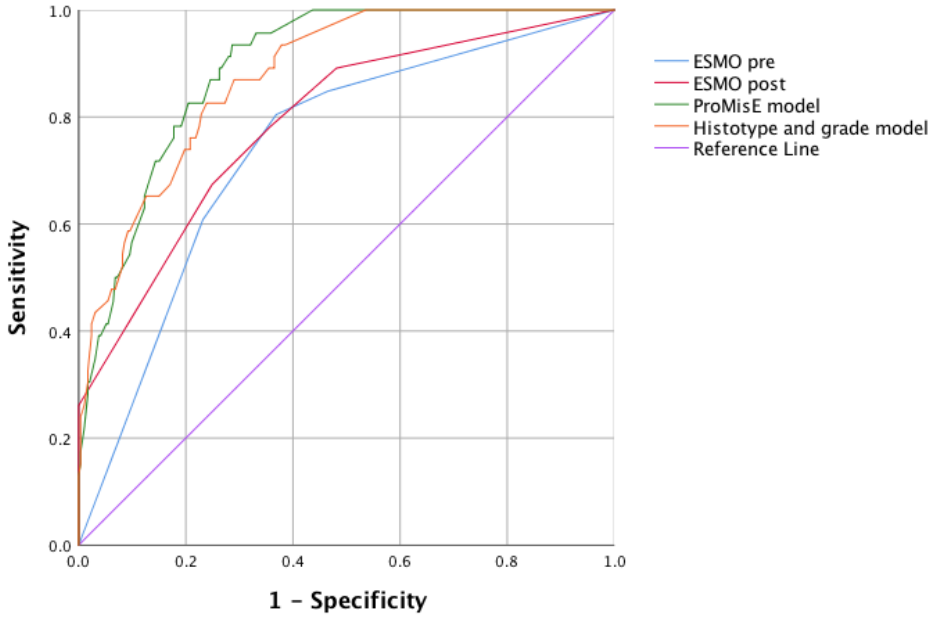

Figure 19. *Receiver operating characteristic curves of predictive ability for recurrence or progression in endometrial cancer (n = 339).*
ESMO pre (preoperative ESMO classification): AUC 0.74 (95% CI 0.67—0.82)
ESMO post (postoperative ESMO classification): AUC 0.79 (95% CI 0.72—0.86)
Histotype and grade model: AUC 0.88 (95% CI 0.83—0.92)
ProMisE model: AUC 0.89 (95% CI 0.85—0.93)

5 DISCUSSION

In this thesis on ultrasound assessment and risk prediction in women with endometrial cancer, we report that preoperative ultrasound staging is best performed by ultrasound experts, as they assess cervical stromal invasion with greater accuracy and interobserver reproducibility than gynecologists (**Study I**), that tumors with presence of the MELF pattern are more than twice as likely to have more advanced stage (≥ IB) and lymph node metastases but that the MELF pattern does not affect the diagnostic accuracy to assess myometrial invasion by ultrasound (**Study II**), that the individual risk of lymph node metastases can be reliably estimated by a preoperative risk prediction model with variables from endometrial biopsy results, clinical and ultrasound characteristics (**Study III**) and that demographic, sonographic and ProMisE variables have the potential to predict the risk of recurrence or progression already before surgery (**Study IV**).

5.1 ULTRASOUND IN THE PREDICTION OF DEEP MYOMETRIAL INVASION AND CERVICAL STROMAL INVASION

Why is it important to accurately predict deep myometrial invasion and cervical stromal invasion before surgery in women with endometrial cancer? Deep myometrial invasion is predictive of lymph node metastases[5, 10, 13-16, 18-22, 24, 25], recurrence and survival[7, 8] and presence of cervical stromal invasion confers a higher stage, associated with worse survival[7]. Because of the increased risk of adverse outcome, preoperative assessment of deep myometrial invasion and cervical stromal invasion is included in preoperative risk classification[27], contributing to the decision on lymphadenectomy. If the preoperative assessment is incorrect, it affects the risk classification with subsequent risk of under- or overtreatment.

Deep myometrial invasion and cervical stromal invasion in **Study I** was assessed subjectively, as subjective assessment is associated with better performance than the use of measurement techniques[54-56]. Reported sensitivities and specificities were in the lower range of other publications (Table 2), most likely due to the fact that the ultrasound experts could not perform live examinations. Had the primary aim been diagnostic accuracy and not interobserver reproducibility, another study design would have been warranted.

We reported greater diagnostic accuracy and interobserver reproducibility in the assessment of cervical stromal invasion by ultrasound experts compared to gynecologists (Table 8 and Table 9), and the recommendation that ultrasound staging is best performed by ultrasound experts has been implemented in international guidelines[27]. No difference was seen between ultrasound experts and gynecologists in the assessment of deep myometrial invasion (Table 8 and Table 9). Possibly could dedicated training programs for gynecologists raise their prediction of cervical stromal invasion to that of ultrasound experts, which could be valuable in areas without access to ultrasound experts and aid in the referral of women with high-risk cancer to tertiary cancer centers for surgery.

Though obesity may limit the possibility to assess the endometrium, by putting the uterus in the upright position[39], BMI did not affect correct assessment of neither deep myometrial invasion nor cervical stromal invasion, which is in agreement with a publication on factors associated with staging error, where neither BMI nor uterine position or image quality were associated with over- or underestimation[63].

According to **Study II**, deep myometrial invasion and cervical stromal invasion was approximately 2—3 times more likely in tumors with the MELF pattern, compared to tumors with conventional myometrial invasion. However, the presence of the MELF pattern did not affect the preoperative ultrasound staging, as sensitivity and specificity for deep myometrial invasion did not differ in tumors with and without presence of the MELF pattern (Table 10). The higher PPV and the lower NPV in tumors with the presence of the MELF pattern is consistent with their increased likelihood of deep myometrial invasion. That significance for the MELF pattern was not reached in multivariable analysis modeling lymph node metastases was likely due to the fact that the study was not primarily designed for this outcome, and was probably underpowered for this analysis, as only 348 women in the study underwent lymphadenectomy.

In **Study IV**, deep myometrial invasion was significantly more common in tumors with AP diameter ≥ 2 cm, verifying that the risk of deep myometrial invasion increases with tumor size[12, 44, 56, 69, 175].

A recent publication shows that the addition of dynamic contrast-enhanced ultrasound improved the detection of deep myometrial invasion and cervical stromal invasion without any increase in the false positive rate[176] and hence has the possibility to improve preoperative risk classification.

5.2 THE CHALLENGE OF CORRECT PREDICTION OF LYMPH NODE METASTASES

Diagnostic imaging modalities alone have been shown to predict lymph node metastases with high specificity but low sensitivity (ultrasound: 100% vs. 33%[58], MRI: 95% vs. 59%[76], PET/CT: 93% vs. 74%[52]). Several known prognostic factors, such as grade, stage, depth of myometrial invasion and tumor size are predictive of the risk of having lymph node metastases[5-7], and current risk classification systems (Table 4) and preoperative risk prediction models (Table 5) combine such prognostic factors, with the aim of improved risk assessment.

Ultrasound is already an established modality in the preoperative risk classification for women with endometrial cancer, in combination with information on histotype and grade from preoperative biopsy[27]. In **Study III**, we assessed how ultrasound, together with variables from endometrial biopsy and clinical characteristics can predict the individual risk of lymph node metastases before surgery, by developing a risk prediction model on a large prospective cohort (n = 1501). Individual risk assessment enables individualized decision making, as higher risks may be deemed acceptable in older, sicker women than in younger,

relatively healthy women. The model performed better than risk classification using ultrasound and biopsy, as recommended in international guidelines[27].

Though grade is an established risk factor for lymph node metastases[5-7] and is included in the major risk classification systems (Table 4), it had no prognostic significance when adjusting for the other model predictors (Table 12). When replacing grade according to biopsy with grade according to hysterectomy, grade did prove to be an independent predictor (grade 1—2 vs. grade 3: OR 1.9, 95% CI 1.2—2.9). This supports the findings of a Meta-analysis[107] that there is only moderate agreement on tumor grade between preoperative biopsy and the hysterectomy specimen. This, together with the limited interobserver reproducibility for grade[78, 102, 103], limits its use in preoperative risk assessment.

The model risk threshold is flexible, and can be adjusted depending on the clinical context, as opposed to those models with fixed risk groups[25, 145, 146, 149]. When choosing the risk threshold, one must decide what is more important: to find those with lymph node metastases (high sensitivity) or to avoid unnecessary lymphadenectomies (high specificity). An ideal risk model would give both at the same time.

Most preoperative risk classification systems and risk prediction models aim at identifying a low-risk group, where lymphadenectomy can be safely omitted[152]. The definition of what "low risk" is varies with models. Our model identified only 10% of the study cohort as having a low risk of lymph node metastases (defined as < 5%). Ideally, this group would have been larger. At the same time, results from leave-center-out cross validation indicate that the model is well calibrated, and that the predicted risks correspond well to the observed frequencies of lymph node metastases, which indicate that actually not more than 10% of women might have a risk lower than 5%. This needs to be established in an external validation cohort.

Published preoperative risk prediction models for lymph node metastases have all been developed on smaller (n = 110—360) retrospective cohorts, differ in FIGO staging system (FIGO 1988 vs. FIGO 2009), use a vast variety of variables, use various modes of imaging (ultrasound vs. MRI), define "low risk" differently and have different target populations (endometrioid cancer vs. endometrioid or non-endometrioid cancer, and tentative stage IA vs. I—II vs. I-IV) (Table 5). The only way to compare the predictive performance of our model to that of the others would be in an independent external validation study. If external evaluation confirms the predictive ability of our model, it could be made more user-friendly by integration into ultrasound machines or apps.

The use of sentinel node biopsy will bring information on lymph node status on most women, also those at low risk, previously not eligible for lymphadenectomy. This limits the need for multiple imputation of lymph node status in the development of future risk prediction models, and could possibly result in models with higher predictive ability.

5.3 PREDICTING RECURRENCE BEFORE SURGERY

Preoperative risk assessment is normally used to estimate the risk of lymph node metastases, whereas the risk of adverse outcome is assessed from postoperative risk assessment, on the basis of hysterectomy variables and surgical stage, known only after performed surgery. Would it be an advantage if we could predict the risk of recurrence already before surgery?

ProMisE might constitute a more objective variable than histotype and grade, by being based on the presence or absence of a protein or a mutation. In comparison to grade[107], ProMisE is associated with a high concordance between preoperative biopsy and hysterectomy specimen[133, 135, 136], making it potentially suitable for preoperative risk assessment.

In **Study IV,** we explored the possibility to use ultrasound, clinical characteristics and ProMisE to predict tumor recurrence or progression in the preoperative setting. We found that a model including demographic (age ≥ 65 years, waist circumference ≥ 88 cm), sonographic (tumor extension and ultrasound tumor AP diameter ≥ 2 cm) and ProMisE variables predicted the risk of recurrence or progression with comparable ability as the corresponding model with histotype and grade, and with higher ability than the ESMO classification (Figure 19), and that non-p53 abn status and ultrasound tumor AP diameter < 2 cm identified a large group of women (48%) at very low risk of adverse outcome (1.8%) (Figure 18). We could confirm p53 abn as an adverse marker, with an almost four times as large risk of recurrence or progression (HR 3.9, 95% CI 1.3—11.1), when adjusted for age, waist circumference, histotype and grade and ultrasound tumor extension, tumor size, endometrial-myometrial junction, color score and vascular pattern (Table 14) and with worse survival outcomes.

To the best of our knowledge, this is the first time an association between increased waist circumference and risk of tumor recurrence or progression has been shown (waist circumference ≥ 88 cm: adjusted HR 2.6, 95% CI 1.2 —5.6) (Table 14). In contrast, we found no increased risk with increasing BMI, which might indicate that the body constitution matters, with an increased risk of adverse outcome in abdominal adiposity.

The conclusions that can be drawn from Study IV must be viewed in relation to the fact that ProMisE was analyzed on the hysterectomy specimen and used as a proxy for ProMisE on preoperative biopsy, which could affect the results, even if high concordance between biopsy and hysterectomy specimen has been reported repeatedly[133, 135, 136].

5.4 YES, SIZE MATTERS

Tumor size on MRI[12, 175], hysterectomy specimen[5, 6, 10, 11, 13, 15, 17-19, 23, 24] and ultrasound[66, 69], is predictive of deep myometrial invasion[69, 175], high-risk disease[66], lymph node metastases[5, 6, 10-13, 15, 17-19, 23, 24, 69, 175] and recurrence and survival[6, 11, 175] and poses an important prognostic factor in endometrial cancer. In spite of this, tumor size is not included in the ESMO classification[27], the GOG-99 criteria[140], the SEPAL criteria[38] or the PORTEC 1 criteria[139], as opposed to the Mayo criteria[6] and the Mayo-modified criteria[143].

In **Study III**, we verified that ultrasound tumor size is predictive of lymph node metastases, as a 0.25 increase in AP tumor/uterine diameter ratio increased the odds of lymph node metastases with 49% (adjusted OR 1.49, 95% CI 1.13—1.97), when all other variables were taken into account (Table 12).

In **Study IV**, larger tumor size, as defined by AP diameter ≥ 2 cm, was an adverse factor, associated with deep myometrial invasion, lymph node metastases, worse survival and a higher risk of recurrence or progression, also among women with preoperative ESMO low risk. The risk of recurrence or progression was almost five times as large for tumors with AP diameter ≥ 2 cm (HR 4.7, 95% CI 1.8—12.4), when adjusted for age, waist circumference, histotype and grade, ProMisE and ultrasound tumor extension, endometrial-myometrial junction, endometrial morphology, color score and vascular pattern (Table 14), verifying that tumor size is predictive of adverse outcome and showing that ultrasound can be used to assess this adverse factor already before surgery.

5.5 WHY INTEROBSERVER REPRODUCIBILITY IS IMPORTANT

It does not matter how accurate a diagnostic test is, if users cannot agree on the result. Since **Study I**, an additional interobserver reproducibility study on ultrasound staging has been published, and showes that 2D ultrasound predicts deep myometrial invasion and cervical stromal invasion with higher interobserver reproducibility than 3D volume contrast imaging (deep myometrial invasion: κ 0.41 vs. κ 0.31 and cervical stromal invasion: κ 0.55 vs. κ 0.45)[177].

When summarizing the results of reproducibility studies on endometrial cancer staging, for the assessment of myometrial invasion, interobserver reproducibility has been found to be moderate for ultrasound[177, 178], fair to good for MRI[96-98] and good to very good for histopathology[99, 101]. For the assessment of cervical stromal invasion, interobserver reproducibility has been found to be moderate for ultrasound[177, 178], moderate to good for MRI[96, 97] and moderate for histopathology[100]. Interobserver reproducibility has been found to be good to very good for histotype[78] and fair to good for grade[78, 102]. Agreement between preoperative biopsy and histotype has been found to be very good for endometrioid cancer[107], non-endometrioid cancer[107] and ProMisE[133, 136], but only moderate for grade[107]. Hence, there are limitations in interobserver reproducibility in preoperative imaging, in histopathological classification and staging and in the agreement between preoperative biopsy and final diagnosis. Based on these findings, it can be argued that risk assessment should not be based on grade and that new systems, such as the use of molecular markers, should be evaluated, both for risk assessment and classification of endometrial cancer.

5.6 WHAT IS THE ROLE OF ULTRASOUND IN THE SENTINEL NODE ERA?

Sentinel node biopsy has emerged as an alternative to complete lymphadenectomy[26], to obtain staging information while reducing morbidity. The risk of lymphedema after lymphadenectomy is 18%—24%[179, 180], while only 1.3% using sentinel node biopsy[179]. According to two meta-analyzes, the sensitivity of sentinel node biopsy to detect metastases

was 94% (95% CI 91—96)[181] vs. 96% (95% CI 91—98)[182] and the NPV 100% (95% CI 99—100)[181] vs. 100% (95% CI not stated)[182]. However, bilateral pelvic sentinel node detection rate was only 61% (95% CI 56—66)[181] vs. 50% (95% CI 44—56)[182], resulting in a need for additional bilateral or side-specific lymphadenectomy to obtain complete surgical staging in the cases with mapping failure. Also, sentinel node biopsy is not accessible everywhere.

The risk prediction model in **Study III** can be used to decide when to perform sentinel node biopsy or lymphadenectomy, depending on the predicted risk of lymph node metastases. It can also be used as a preoperative complement to sentinel node biopsy, to guide whether or not to continue with uni- or bilateral lymphadenectomy in the case of mapping failure.

In **Study IV** we could identify a very low-risk group for recurrence or progression (1.8%), constituting half of the study population (48%) by assessing ultrasound tumor AP diameter (< 2 cm) and p53 status (non-p53 abn). It can be argued that not even sentinel node biopsy might be necessary in such very low-risk cases.

Theoretically, if preoperative risk assessment was good enough to correctly identify all women with lymph node metastases, both sentinel node biopsy and lymphadenectomy could be omitted. Moreover, the value of lymph node assessment on survival is uncertain. If studies would show that knowledge on lymph node status after lymph node dissection has no value in tailoring the adjuvant treatment, both preoperative risk assessment and sentinel node biopsy/lymphadenectomy could be omitted. However, ultrasound would still have value in the preoperative work up, by identifying women with extrauterine spread, where modified surgery is warranted, or in the discrimination of primary cervical cancer or synchronous ovarian cancer, where tru-cut biopsy can be diagnostic.

6 METHODOLOGICAL CONSIDERATIONS

6.1 STUDY DESIGN

The major strength of the included studies (**Study I—IV**) is the origin of study participants, from the largest prospective multicenter cohort of endometrial cancer to date. Ultrasound was performed by expert examiners, using a standardized ultrasound protocol, resulting in high quality ultrasound data. The prospective gathering of pre-defined demographic variables resulted in comprehensive demographic data. The use of data capture software assured completeness of records on demography, ultrasound and surgery, as the software did not allow saving of incomplete forms. The size and the multicenter nature of the cohort increase the likelihood that results are generalizable.

A limitation of **Study I** was the use of off-line videoclips instead of live ultrasound examinations. As an identical material is required to evaluate interobserver reproducibility, all ultrasound experts and gynecologists needed to assess the same women. Granted the number of assessors, from various countries, live examination would have been ethically questionable and not practically feasible.

The off-line setting, with videoclips collected by an ultrasound expert, might have facilitated the assessment for the gynecologists, by guiding them to the region of interest. In spite of this possible advantage, gynecologists still had lower interobserver reproducibility than ultrasound experts in the assessment of cervical stromal invasion.

A limitation of **Study II** is the absence of a centralized pathology slide review for the presence of MELF. However, international transportation of pathology slides from various counties had been difficult to perform and was not included in the approval by the Ethics Committee. Also, the multicenter nature of the data was thought to increase the generalizability of the results.

A limitation of **Study III** is the missing of outcome data on half of the model development population. However, due to guidelines on lymphadenectomy at the time, and the absence of sentinel node biopsy for lymph node data also in low-risk women, complete outcome data on a general population had not been obtainable.

A limitation of **Study IV** was analysis of ProMisE on the hysterectomy specimen and not the preoperative biopsy. Though publications report high concordance between ProMisE analysis on hysterectomy and biopsy specimen, and all other variables were obtained from preoperative data, we do not know if and how results could have varied had ProMisE analysis been performed on the biopsy specimen, which limits the conclusions that can be drawn from this study.

6.2 SELECTION BIAS

Selection bias occurs when the association of an exposure and an outcome differs between subjects included in the study and the overall population, resulting in a sample that does not accurately reflect the target population.

There is no registration on how many women that were eligible for inclusion in the IETA 4 study cohort, constituting the basis for all studies **(Study I—IV)** in this thesis. Some women with presumed cancer limited to the corpus uteri might not have been referred to preoperative ultrasound staging, with a possible over representation of women with grade 3 endometrial cancer or non-endometrioid cancer on preoperative biopsy, where lymphadenectomy would have been indicated regardless of the ultrasound result, and of women to old or to sick for lymphadenectomy, creating a possible selection bias towards a low-risk sample.

Within the IETA 4 study cohort, lymphadenectomy was more often performed in women classified as high risk for lymph node metastases, leading to a selection bias where women with known lymph node status constitute a high-risk sample. Indeed, women subject to lymphadenectomy in **Study III** had more cases of deep myometrial invasion on ultrasound, more often grade 3 endometrioid or non-endometrioid cancer and higher tumor stage, compared to those not subject to lymphadenectomy. A model developed on only the cases with complete outcome data would have been subject to bias induced by these systematic differences. To adjust for systematic differences and to create a risk prediction model applicable on all women, and not only those with high risk, lymph node status was multiply imputed when missing.

6.3 CLASSIFICATION BIAS

Classification bias, also called measurement or information bias, results from improper measurement of exposure or outcome variables. The time between ultrasound examination and surgery can possibly have created a classification bias, as there is a chance of disease progression between the two events, with subsequent underestimation of ultrasound to correctly classify cases. The effect of this was mitigated by the exclusion of all women from the IETA 4 study cohort with three months or more between ultrasound examination and surgery.

Deep myometrial invasion and cervical stromal invasion in the hysterectomy specimen is assessed through subjective evaluation, with varying interobserver reproducibility. This indicates the possibility of classification bias in **Study I** and **Study II**, where cases with possible wrong classification of deep myometrial invasion and cervical stromal invasion according to histopathology still were used as gold standard in the evaluation of ultrasound staging.

In **Study II**, possible differences in evaluation of MELF status within and between centers were mitigated through the use of reference pathologists only and by text and image examples, still the presence of the MELF pattern varied between centers. As study

populations did not differ regarding tumor stage, cervical stromal invasion, deep myometrial invasion or lymph node metastases between centers more or less prone to diagnose the MELF pattern, the difference in MELF status could be attributed at least partly to classification bias.

Tumor size measurements (**Study II-IV**) should be interpreted with some caution, as most women had undergone various endometrial biopsy procedures before ultrasound examination, with possible subsequent underestimation of actual tumor size.

Women subject to lymphadenectomy in **Study III** had higher tumor stage, consistent with lymphadenectomy being performed more often in women with high-risk disease. However, stage migration occurs in women with presumed endometrial cancer limited to the uterus, when lymphadenectomy is performed and reveals lymph node metastases, compared to if lymphadenectomy had not been performed and the existing lymph node metastases had not been found. It is possible that the difference seen in tumor stage between women subject to and not subject to lymphadenectomy may also have been affected by misclassification.

The histopathologic assessment of lymph nodes in **Study II** and **Study III** was performed through conventional sectioning and not through ultrastaging. Possibly could more cases with lymph node metastases have been found if ultrastaging had been used, providing better power to find a possible adjusted association between the MELF pattern and the presence of lymph node metastases (**Study II**) and enabling the use of more variables in the model building (**Study III**) without increased risk of overfitting.

6.4 CONFOUNDING

Confounding occurs when the apparent association between an explanatory variable and the outcome is affected by the relationship of a third variable, which is associated with the explanatory variable and casually related to the outcome[183]. A major challenge when building logistic regression models is to select which variables to include. A model with many variables presents less statistical power, and significant relationship between an explanatory variable and the outcome may be missed. Also, the results, including significant existing associations or not, depend on which level within a variable that is chosen as reference group[166]. In a RCT, the randomization process compensates for known and unknown confounders by dividing them evenly in the groups.

When assessing the relationship between the explanatory variables and the outcome in **Study II** (MELF and higher stage or lymph node metastases), **Study III** (preoperative variables and lymph node metastases) and **Study IV** (preoperative variables and recurrence or progression), constituting cohort studies, there is always the possibility that confounding variables affected the outcome, and that variables not included in the regression models could have posed an important effect on the outcome.

6.5 EXTERNAL VALIDITY

External validity refers to the extent to which the results of a study can be generalized to another population. The results of **Study I** are applicable only to off-line settings, whereas the results of **Study II—IV** are applicable to settings where ultrasound is performed by ultrasound experts, using high-end equipment.

In **Study III**, we excluded women originating from a center with a screening population from the model development cohort, to increase the generalizability of the model developing cohort to the general population. When creating a risk prediction model, like in **Study III**, an important consideration is the complexity of the model relative to the available sample size. Overly complex models are often overfitted, and perform well on the model development data but poorly on new data. Except for overfitting, another threat to external validity is "apparent" model validation, where the predictive performance has been assessed on the same data set that was used for model development. In **Study III**, overfitting was prevented by selecting predictors of lymph node metastases a priori instead of data-driven, by using a limited set of predictors to yield 10 events per predictor, and by using a heuristic shrinkage factor to get the final prediction equation. The large sample size, the multi-center design and the results from "leave-center-out-cross-validation", mimicking external validation, suggests good generalizability of the model.

When developing a risk prediction model, the development cohort should be representative of the intended target population for the model, unlike the case in models created on retrospective cohorts, consisting only of women that were subject to lymphadenectomy. The multiple imputation used in **Study III** enabled model development on all women and limited the effect of selection bias. However, the result of multiple imputation, and hence the external validity of the risk prediction model developed on the imputed cohort, depends on how well the imputation was performed. Though professional statisticians performed the multiple imputation, using established methods that were aided by the availability of auxiliary variables such as hysterectomy findings, it can always be argued that not all factors related to the outcome were taken into consideration. External validation on an independent cohort, preferably with known sentinel node outcomes in cases without lymphadenectomy, is warranted before the model can be used in clinical practice.

Study IV is based on a single center cohort from a general population, as opposed to a high-risk population from a tertiary referral center. The external validity on a general population is assumed to be good, as we have reproduced results from a published validation study in a population-based cohort[133].

7 CONCLUSION

Study I:

- Gynecologists and ultrasound experts assessed deep myometrial invasion with comparable diagnostic accuracy and interobserver reproducibility.
- Compared to gynecologists, ultrasound experts assessed cervical stromal invasion with greater diagnostic accuracy and interobserver reproducibility.
- Preoperative ultrasound staging in endometrial cancer is best performed by ultrasound experts.

Study II:

- Tumors with the MELF pattern were slightly larger, with a higher color score and more often the multiple multifocal vascular pattern on preoperative ultrasound.
- Presence of the MELF pattern did not affect the diagnostic accuracy of deep myometrial invasion by preoperative ultrasound.
- Tumors with the MELF pattern were more than twice as likely to have more advanced stage (≥IB) and lymph node metastases.

Study III:

- A risk prediction model with variables from endometrial biopsy results, clinical and ultrasound characteristics can reliably estimate the individual risk of lymph node metastases before surgery in women with endometrial cancer.
- The risk prediction model is superior to risk classification by endometrial biopsy alone or in combination with ultrasound.

Study IV:

- The ProMisE p53 abn subtype is an adverse prognostic marker, associated with older age, larger tumors on ultrasound, non-endometrioid cancer, higher stage, tumor recurrence or progression and worse survival.
- Ultrasound tumor size < 2 cm and non-p53 abn status have the potential to identify women at very low risk of adverse outcome.
- A model with demographic, sonographic and ProMisE variables had higher ability to predict recurrence or progression than the ESMO classification.

8 FUTURE PERSPECTIVES

The role of a classification system for endometrial cancer based on molecular markers should be further explored, to obtain a classification with higher interobserver reproducibility and higher agreement between preoperative biopsy and hysterectomy specimen than the current system based on histotype and grade.

An external validation study of published preoperative risk models on a large prospective cohort, with complete data in all women of all variables included in the models eligible for validation, would answer which model that best predicts the risk of lymph node metastases and establish if our model can be put into use in clinical routine.

Further studies are warranted to assess if incorporation of molecular markers to other preoperative variables from demography, endometrial biopsy and imaging can improve the prediction of lymph node metastases and adverse outcome in risk models and classification systems. These studies should be preceded by interobserver reproducibility studies on eligible predictive variables, to obtain robust risk models and classification systems.

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10 REFERENCES

1. Socialstyrelsen. Cancer i siffror 2018. www.socialstyrelse.se
2. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial cancer. *Lancet*. 2016;387:1094-1108.
3. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. *Lancet*. 2005;366:491-505.
4. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer*. 2004;4:579-591.
5. Creasman WT, Morrow CP, Bundy BN, Homesley HD, Graham JE, Heller PB. Surgical pathologic spread patterns of endometrial cancer. A Gynecologic Oncology Group Study. *Cancer*. 1987;60:2035-2041.
6. Mariani A, Webb MJ, Keeney GL, Haddock MG, Calori G, Podratz KC. Low-risk corpus cancer: is lymphadenectomy or radiotherapy necessary? *Am J Obstet Gynecol*. 2000;182:1506-1519.
7. Creasman WT, Odicino F, Maisonneuve P, Quinn MA, Beller U, Benedet JL, et al. Carcinoma of the corpus uteri. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. *Int J Gynaecol Obstet*. 2006 Nov;95 Suppl 1:S105-43.
8. Sorbe B. Predictive and prognostic factors in definition of risk groups in endometrial carcinoma. *ISRN Obstet Gynecol*. 2012;2012:325790.
9. Hamilton CA, Cheung MK, Osann K, Chen L, Teng NN, Longacre TA, et al. Uterine papillary serous and clear cell carcinomas predict for poorer survival compared to grade 3 endometrioid corpus cancers. *Br J Cancer*. 2006;94:642-646.
10. Kamura T, Yahata H, Shigematsu T, Ogawa S, Amada S, Kaku T, et al. Predicting pelvic lymph node metastasis in endometrial carcinoma. *Gynecol Oncol*. 1999;72:387-391.
11. Mahdi H, Munkarah AR, Ali-Fehmi R, Woessner J, Shah SN, Moslemi-Kebria M. Tumor size is an independent predictor of lymph node metastasis and survival in early stage endometrioid endometrial cancer. *Arch Gynecol Obstet*. 2015;292:183-190.
12. Bourgioti C, Chatoupis K, Tzavara C, Antoniou A, Rodolakis A, Moulopoulos LA. Predictive ability of maximal tumor diameter on MRI for high-risk endometrial cancer. *Abdom Radiol (NY)*. 2016;41:2484-2495.
13. AlHilli MM, Podratz KC, Dowdy SC, Bakkum-Gamez JN, Weaver AL, McGree ME, et al. Risk-scoring system for the individualized prediction of lymphatic dissemination in patients with endometrioid endometrial cancer. *Gynecol Oncol*. 2013;131:103-108.
14. Bendifallah S, Genin AS, Naoura I, Chabbert Buffet N, Clavel Chapelon F, Haddad B, et al. A nomogram for predicting lymph node metastasis of presumed stage I and II endometrial cancer. *Am J Obstet Gynecol*. 2012;207:197 e1-8.
15. Milam MR, Java J, Walker JL, Metzinger DS, Parker LP, Coleman RL, et al. Nodal metastasis risk in endometrioid endometrial cancer. *Obstet Gynecol*. 2012;119:286-292.
16. Zhang C, Wang C, Feng W. Clinicopathological risk factors for pelvic lymph node metastasis in clinical early-stage endometrioid endometrial adenocarcinoma. *Int J Gynecol Cancer*. 2012;22:1373-1377.
17. Schink JC, Rademaker AW, Miller DS, Lurain JR. Tumor size in endometrial cancer. *Cancer*. 1991;67:2791-2794.
18. Meydanli MM, Aslan K, Oz M, Muftuoglu KH, Yalcin I, Engin-Ustun Y. A novel multivariable prediction model for lymphatic dissemination in endometrioid endometrial cancer: The lymph node Metastasis Risk Index. *Eur J Obstet Gynecol Reprod Biol*. 2019;240:310-315.
19. Pollom EL, Conklin CM, von Eyben R, Folkins AK, Kidd EA. Nomogram to Predict Risk of Lymph Node Metastases in Patients With Endometrioid Endometrial Cancer. *Int J Gynecol Pathol*. 2016;35:395-401.
20. Gilani S, Anderson I, Fathallah L, Mazzara P. Factors predicting nodal metastasis in endometrial cancer. *Arch Gynecol Obstet*. 2014;290:1187-1193.
21. Kumar S, Mariani A, Bakkum-Gamez JN, Weaver AL, McGree ME, Keeney GL, et al. Risk factors that mitigate the role of paraaortic lymphadenectomy in uterine endometrioid cancer. *Gynecol Oncol*. 2013;130:441-445.
22. Boren T, Lea J, Kehoe S, Miller DS, Richardson D. Lymph node metastasis in endometrioid adenocarcinomas of the uterine corpus with occult cervical involvement. *Gynecol Oncol*. 2012;127:43-46.
23. Turan T, Hizli D, Sarici S, Boran N, Gundogdu B, Karadag B, et al. Is it possible to predict para-aortic lymph node metastasis in endometrial cancer? *Eur J Obstet Gynecol Reprod Biol*. 2011;158:274-279.
24. Lee KB, Ki KD, Lee JM, Lee JK, Kim JW, Cho CH, et al. The risk of lymph node metastasis based on myometrial invasion and tumor grade in endometrioid uterine cancers: a multicenter, retrospective Korean study. *Ann Surg Oncol*. 2009;16:2882-2887.
25. Lee J, Kong TW, Paek J, Chang SJ, Ryu HS. Predicting Model of Lymph Node Metastasis Using Preoperative Tumor Grade, Transvaginal Ultrasound, and Serum CA-125 Level in Patients With Endometrial Cancer. *Int J Gynecol Cancer*. 2016;26:1630-1635.
26. Rossi EC, Kowalski LD, Scalici J, Cantrell L, Schuler K, Hanna RK, et al. A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): a multicentre, prospective, cohort study. *Lancet Oncol*. 2017 Jan 31.
27. Colombo N, Creutzberg C, Amant F, Bosse T, Gonzalez-Martin A, Ledermann J, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: diagnosis, treatment and follow-up. *Ann Oncol*. 2016;27:16-41.
28. Chan JK, Kapp DS. Role of complete lymphadenectomy in endometrioid uterine cancer. *Lancet Oncol*. 2007;8:831-841.
29. Karalok A, Turan T, Basaran D, Turkmen O, Comert Kimyon G, Tulunay G, et al. Lymph Node Metastasis in Patients With Endometrioid Endometrial Cancer: Overtreatment Is the Main Issue. *Int J Gynecol Cancer*. 2017;27:748-753.

30. Mariani A, Dowdy SC, Cliby WA, Gostout BS, Jones MB, Wilson TO, et al. Prospective assessment of lymphatic dissemination in endometrial cancer: a paradigm shift in surgical staging. *Gynecol Oncol*. 2008;109:11-18.

31. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. *Int J Gynaecol Obstet*. 2009;105:103-104.

32. Sobin L, Gospodarowicz M, Wittekind C. TNM Classification of Malignant Tumors. 7th ed: John Wiley & Sons: New York; 2009.

33. Boronow RC, Morrow CP, Creasman WT, Disaia PJ, Silverberg SG, Miller A, et al. Surgical staging in endometrial cancer: clinical-pathologic findings of a prospective study. *Obstet Gynecol*. 1984;63:825-832.

34. Mikuta JJ. International Federation of Gynecology and Obstetrics staging of endometrial cancer 1988. *Cancer*. 1993;71:1460-1463.

35. Frost JA, Webster KE, Bryant A, Morrison J. Lymphadenectomy for the management of endometrial cancer. *Cochrane Database Syst Rev*. 2017;10:CD007585.

36. Benedetti Panici P, Basile S, Maneschi F, Alberto Lissoni A, Signorelli M, Scambia G, et al. Systematic pelvic lymphadenectomy vs. no lymphadenectomy in early-stage endometrial carcinoma: randomized clinical trial. *J Natl Cancer Inst*. 2008;100:1707-1716.

37. Kitchener H, Swart AM, Qian Q, Amos C, Parmar MK. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study. *Lancet*.2009;373:125-136.

38. Todo Y, Kato H, Kaneuchi M, Watari H, Takeda M, Sakuragi N. Survival effect of para-aortic lymphadenectomy in endometrial cancer (SEPAL study): a retrospective cohort analysis. *Lancet*. 2010;375:1165-1172.

39. Epstein E, Blomqvist L. Imaging in endometrial cancer. *Best Pract Res Clin Obstet Gynaecol*. 2014;28:721-739.

40. Leone FP, Timmerman D, Bourne T, Valentin L, Epstein E, Goldstein SR, et al. Terms, definitions and measurements to describe the sonographic features of the endometrium and intrauterine lesions: a consensus opinion from the International Endometrial Tumor Analysis (IETA) group. *Ultrasound Obstet Gynecol*. 2010;35:103-112.

41. Fischerova D. Ultrasound scanning of the pelvis and abdomen for staging of gynecological tumors: a review. *Ultrasound Obstet Gynecol*. 2011;38:246-266.

42. Gordon AN, Fleischer AC, Reed GW. Depth of myometrial invasion in endometrial cancer: preoperative assessment by transvaginal ultrasonography. *Gynecol Oncol*. 1990;39:321-327.

43. Karlsson B, Norstrom A, Granberg S, Wikland M. The use of endovaginal ultrasound to diagnose invasion of endometrial carcinoma. *Ultrasound Obstet Gynecol*. 1992;2:35-39.

44. Mascilini F, Testa AC, Van Holsbeke C, Ameye L, Timmerman D, Epstein E. Evaluating myometrial and cervical invasion in women with endometrial cancer: comparing subjective assessment with objective measurement techniques. *Ultrasound Obstet Gynecol*. 2013;42:353-358.

45. Alcazar JL, Galvan R, Albela S, Martinez S, Pahisa J, Jurado M, et al. Assessing myometrial infiltration by endometrial cancer: uterine virtual navigation with three-dimensional US. *Radiology*. 2009;250:776-783.

46. van Doorn HC, van der Zee AG, Peeters PH, Kroeks MV, van Eijkeren MA. Preoperative selection of patients with low-stage endometrial cancer at high risk of pelvic lymph node metastases. *Int J Gynecol Cancer*. 2002;12:144-148.

47. De Smet F, De Brabanter J, Van den Bosch T, Pochet N, Amant F, Van Holsbeke C, et al. New models to predict depth of infiltration in endometrial carcinoma based on transvaginal sonography. *Ultrasound Obstet Gynecol*. 2006;27:664-671.

48. Savelli L, Ceccarini M, Ludovisi M, Fruscella E, De Iaco PA, Salizzoni E, et al. Preoperative local staging of endometrial cancer: transvaginal sonography vs. magnetic resonance imaging. *Ultrasound Obstet Gynecol*. 2008;31:560-566.

49. Ozdemir S, Celik C, Emlik D, Kiresi D, Esen H. Assessment of myometrial invasion in endometrial cancer by transvaginal sonography, Doppler ultrasonography, magnetic resonance imaging and frozen section. *Int J Gynecol Cancer*. 2009;19:1085-1090.

50. Akbayir O, Corbacioglu A, Numanoglu C, Goksedef BP, Guraslan H, Akagunduz G, et al. Combined use of preoperative transvaginal ultrasonography and intraoperative gross examination in the assessment of myometrial invasion in endometrial carcinoma. *Eur J Obstet Gynecol Reprod Biol*. 2012;165:284-288.

51. Savelli L, Testa AC, Mabrouk M, Zannoni L, Ludovisi M, Seracchioli R, et al. A prospective blinded comparison of the accuracy of transvaginal sonography and frozen section in the assessment of myometrial invasion in endometrial cancer. *Gynecol Oncol*. 2012;124:549-552.

52. Antonsen SL, Jensen LN, Loft A, Berthelsen AK, Costa J, Tabor A, et al. MRI, PET/CT and ultrasound in the preoperative staging of endometrial cancer - a multicenter prospective comparative study. *Gynecol Oncol*. 2013;128:300-308.

53. Van Holsbeke C, Ameye L, Testa AC, Mascilini F, Lindqvist P, Fischerova D, et al. Development and external validation of new ultrasound-based mathematical models for preoperative prediction of high-risk endometrial cancer. *Ultrasound Obstet Gynecol*. 2014;43:586-595.

54. Alcazar JL, Pineda L, Martinez-Astorquiza Corral T, Orozco R, Utrilla-Layna J, Juez L, et al. Transvaginal/transrectal ultrasound for assessing myometrial invasion in endometrial cancer: a comparison of six different approaches. *J Gynecol Oncol*. 2015;26:201-207.

55. Fruhauf F, Zikan M, Semeradova I, Dundr P, Nemejcova K, Dusek L, et al. The Diagnostic Accuracy of Ultrasound in Assessment of Myometrial Invasion in Endometrial Cancer: Subjective Assessment versus Objective Techniques. *Biomed Res Int*. 2017;2017:1318203.

56. Verbakel JY, Mascilini F, Wynants L, Fischerova D, Testa AC, Franchi D, et al. Validation of ultrasound strategies to assess tumor extension and to predict high-risk endometrial cancer in women from the prospective IETA (International Endometrial Tumour Analysis) 4 cohort. *Ultrasound Obstet Gynecol*. 2019. doi 10.1002/uog.20374

57. Gabrielli S, Marabini A, Bevini M, Linsalata I, Falco P, Milano V, et al. Transvaginal sonography vs. hysteroscopy in the preoperative staging of endometrial carcinoma. *Ultrasound Obstet Gynecol*. 1996;7:443-446.

58. Sawicki W, Spiewankiewicz B, Stelmachow J, Cendrowski K. The value of ultrasonography in preoperative assessment of selected prognostic factors in endometrial cancer. *Eur J Gynaecol Oncol*. 2003;24:293-298.

59. Celik C, Ozdemir S, Kiresi D, Emlik D, Tazegul A, Esen H. Evaluation of cervical involvement in endometrial cancer by transvaginal sonography, magnetic resonance imaging and frozen section. *J Obstet Gynaecol*. 2010;30:302-307.

60. Akbayir O, Corbacioglu A, Numanoglu C, Guleroglu FY, Ulker V, Akyol A, et al. Preoperative assessment of myometrial and cervical invasion in endometrial carcinoma by transvaginal ultrasound. *Gynecol Oncol*. 2011;122:600-603.

61. Ortoft G, Dueholm M, Mathiesen O, Hansen ES, Lundorf E, Moller C, et al. Preoperative staging of endometrial cancer using TVS, MRI, and hysteroscopy. *Acta Obstet Gynecol Scand*. 2013;92:536-545.

62. Christensen JW, Dueholm M, Hansen ES, Marinovskij E, Lundorf E, Ortoft G. Assessment of myometrial invasion in endometrial cancer using three-dimensional ultrasound and magnetic resonance imaging. *Acta Obstet Gynecol Scand*. 2016;95:55-64.

63. Fischerova D, Fruhauf F, Zikan M, Pinkavova I, Kocian R, Dundr P, et al. Factors affecting sonographic preoperative local staging of endometrial cancer. *Ultrasound Obstet Gynecol*. 2014;43:575-585.

64. Soliman PT, Slomovitz BM, Broaddus RR, Sun CC, Oh JC, Eifel PJ, et al. Synchronous primary cancers of the endometrium and ovary: a single institution review of 84 cases. *Gynecol Oncol*. 2004;94:456-462.

65. Epstein E, Van Holsbeke C, Mascilini F, Masback A, Kannisto P, Ameye L, et al. Gray-scale and color Doppler ultrasound characteristics of endometrial cancer in relation to stage, grade and tumor size. *Ultrasound Obstet Gynecol*. 2011;38:586-593.

66. Epstein E, Fischerova D, Valentin L, Testa AC, Franchi D, Sladkevicius P, et al. Ultrasound characteristics of endometrial cancer as defined by International Endometrial Tumor Analysis (IETA) consensus nomenclature: prospective multicenter study. *Ultrasound Obstet Gynecol*. 2018;51:818-828.

67. Timmerman D, Valentin L, Bourne TH, Collins WP, Verrelst H, Vergote I. Terms, definitions and measurements to describe the sonographic features of adnexal tumors: a consensus opinion from the International Ovarian Tumor Analysis (IOTA) Group. *Ultrasound Obstet Gynecol*. 2000;16:500-505.

68. Alcazar JL, Jurado M. Three-dimensional ultrasound for assessing women with gynecological cancer: a systematic review. *Gynecol Oncol*. 2011;120:340-346.

69. Galvan R, Merce L, Jurado M, Minguez JA, Lopez-Garcia G, Alcazar JL. Three-dimensional power Doppler angiography in endometrial cancer: correlation with tumor characteristics. *Ultrasound Obstet Gynecol*. 2010;35:723-729.

70. Armstrong L, Fleischer A, Andreotti R. Three-dimensional volumetric sonography in gynecology: an overview of clinical applications. *Radiol Clin North Am*. 2013;51:1035-1047.

71. Yang T, Tian S, Li Y, Tian X, Wang W, Zhao J, et al. Magnetic Resonance Imaging (MRI) and Three-Dimensional Transvaginal Ultrasonography Scanning for Preoperative Assessment of High Risk in Women with Endometrial Cancer. *Med Sci Monit*. 2019;25:2024-2031.

72. Rodriguez-Trujillo A, Martinez-Serrano MJ, Martinez-Roman S, Marti C, Bunesch L, Nicolau C, et al. Preoperative Assessment of Myometrial Invasion in Endometrial Cancer by 3D Ultrasound and Diffusion-Weighted Magnetic Resonance Imaging: A Comparative Study. *Int J Gynecol Cancer*. 2016;26:1105-1110.

73. Das SK, Niu XK, Wang JL, Zeng LC, Wang WX, Bhetuwal A, et al. Usefulness of DWI in preoperative assessment of deep myometrial invasion in patients with endometrial carcinoma: a systematic review and meta-analysis. *Cancer Imaging*. 2014;14:32.

74. Deng L, Wang QP, Chen X, Duan XY, Wang W, Guo YM. The Combination of Diffusion- and T2-Weighted Imaging in Predicting Deep Myometrial Invasion of Endometrial Cancer: A Systematic Review and Meta-Analysis. *J Comput Assist Tomogr*. 2015;39:661-673.

75. Luomaranta A, Leminen A, Loukovaara M. Magnetic resonance imaging in the assessment of high-risk features of endometrial carcinoma: a meta-analysis. *Int J Gynecol Cancer*. 2015;25:837-842.

76. Bi Q, Chen Y, Wu K, Wang J, Zhao Y, Wang B, et al. The Diagnostic Value of MRI for Preoperative Staging in Patients with Endometrial Cancer: A Meta-Analysis. *Acad Radiol*. 2019 Oct 18.

77. Cicinelli E, Marinaccio M, Barba B, Tinelli R, Colafiglio G, Pedote P, et al. Reliability of diagnostic fluid hysteroscopy in the assessment of cervical invasion by endometrial carcinoma: a comparative study with transvaginal sonography and MRI. *Gynecol Oncol*. 2008;111:55-61.

78. Clarke BA, Gilks CB. Endometrial carcinoma: controversies in histopathological assessment of grade and tumour cell type. *J Clin Pathol*. 2010;63:410-415.

79. Shepherd JH. Revised FIGO staging for gynaecological cancer. *Br J Obstet Gynaecol*. 1989;96:889-892.

80. Lax SF, Kurman RJ, Pizer ES, Wu L, Ronnett BM. A binary architectural grading system for uterine endometrial endometrioid carcinoma has superior reproducibility compared with FIGO grading and identifies subsets of advance-stage tumors with favorable and unfavorable prognosis. *Am J Surg Pathol*. 2000;24:1201-1208.

81. Taylor RR, Zeller J, Lieberman RW, O'Connor DM. An analysis of two versus three grades for endometrial carcinoma. *Gynecol Oncol*. 1999;74:3-6.

82. Scholten AN, Creutzberg CL, Noordijk EM, Smit VT. Long-term outcome in endometrial carcinoma favors a two- instead of a three-tiered grading system. *Int J Radiat Oncol Biol Phys*. 2002;52:1067-1074.

83. Scholten AN, Smit VT, Beerman H, van Putten WL, Creutzberg CL. Prognostic significance and interobserver variability of histologic grading systems for endometrial carcinoma. *Cancer*. 2004;100:764-772.

84. Murray SK, Young RH, Scully RE. Unusual epithelial and stromal changes in myoinvasive endometrioid adenocarcinoma: a study of their frequency, associated diagnostic problems, and prognostic significance. *Int J Gynecol Pathol*. 2003;22:324-333.

85. Hertel JD, Huettner PC, Pfeifer JD. Lymphovascular space invasion in microcystic elongated and fragmented (MELF)-pattern well-differentiated endometrioid adenocarcinoma is associated with a higher rate of lymph node metastasis. *Int J Gynecol Pathol*. 2014;33:127-134.

86. Pavlakis K, Messini I, Vrekoussis T, Panoskaltsis T, Chrysanthakis D, Yiannou P, et al. MELF invasion in endometrial cancer as a risk factor for lymph node metastasis. *Histopathology*. 2011;58:966-973.

87. Stewart CJ, Brennan BA, Leung YC, Little L. MELF pattern invasion in endometrial carcinoma: association with low grade, myoinvasive endometrioid tumours, focal mucinous differentiation and vascular invasion. *Pathology*. 2009;41:454-459.

88. Dogan Altunpulluk M, Kir G, Topal CS, Cetiner H, Gocmen A. The association of the microcystic, elongated and fragmented (MELF) invasion pattern in endometrial carcinomas with deep myometrial invasion, lymphovascular space invasion and lymph node metastasis. *J Obstet Gynaecol*. 2015;35:397-402.

89. Euscher E, Fox P, Bassett R, Al-Ghawi H, Ali-Fehmi R, Barbuto D, et al. The pattern of myometrial invasion as a predictor of lymph node metastasis or extrauterine disease in low-grade endometrial carcinoma. *Am J Surg Pathol*. 2013;37:1728-1736.

90. Joehlin-Price AS, McHugh KE, Stephens JA, Li Z, Backes FJ, Cohn DE, et al. The Microcystic, Elongated, and Fragmented (MELF) Pattern of Invasion: A Single Institution Report of 464 Consecutive FIGO Grade 1 Endometrial Endometrioid Adenocarcinomas. *Am J Surg Pathol*. 2017;41:49-55.

91. Quick CM, May T, Horowitz NS, Nucci MR. Low-grade, low-stage endometrioid endometrial adenocarcinoma: a clinicopathologic analysis of 324 cases focusing on frequency and pattern of myoinvasion. *Int J Gynecol Pathol*. 2012;31:337-343.

92. Ali A, Black D, Soslow RA. Difficulties in assessing the depth of myometrial invasion in endometrial carcinoma. *Int J Gynecol Pathol*. 2007;26:115-123.

93. Han G, Lim D, Leitao MM, Jr., Abu-Rustum NR, Soslow RA. Histological features associated with occult lymph node metastasis in FIGO clinical stage I, grade I endometrioid carcinoma. *Histopathology*. 2014;64:389-398.

94. Roma AA, Rybicki LA, Barbuto D, Euscher E, Djordjevic B, Frauenhoffer E, et al. Risk factor analysis of recurrence in low-grade endometrial adenocarcinoma. *Hum Pathol*. 2015 Oct;46(10):1529-1539.

95. Alcazar JL, Merce LT, Manero MG, Bau S, Lopez-Garcia G. Endometrial volume and vascularity measurements by transvaginal 3-dimensional ultrasonography and power Doppler angiography in stimulated and tumoral endometria: an interobserver reproducibility study. *J Ultrasound Med*. 2005;24:1091-1098.

96. Hori M, Kim T, Murakami T, Imaoka I, Onishi H, Nakamoto A, et al. MR imaging of endometrial carcinoma for preoperative staging at 3.0 T: comparison with imaging at 1.5 T. *Journal of magnetic resonance imaging : JMRI*. 2009;30:621-630.

97. Haldorsen IS, Husby JA, Werner HM, Magnussen IJ, Rorvik J, Helland H, et al. Standard 1.5-T MRI of endometrial carcinomas: modest agreement between radiologists. *European radiology*. 2012;22:1601-1611.

98. Husby JA, Salvesen OO, Magnussen IJ, Trovik J, Bjorge L, Salvesen HB, et al. Tumour apparent diffusion coefficient is associated with depth of myometrial invasion and is negatively correlated to tumour volume in endometrial carcinomas. *Clin Radiol*. 2015;70:487-494.

99. Nedergaard L, Jacobsen M, Andersen JE. Interobserver agreement for tumour type, grade of differentiation and stage in endometrial carcinomas. *Apmis*. 1995;103:511-518.

100. McCluggage WG, Hirschowitz L, Wilson GE, Oliva E, Soslow RA, Zaino RJ. Significant variation in the assessment of cervical involvement in endometrial carcinoma: an interobserver variation study. *Am J Surg Pathol*. 2011;35:289-294.

101. van der Putten LJ, van de Vijver K, Bartosch C, Davidson B, Gatius S, Matias-Guiu X, et al. Reproducibility of measurement of myometrial invasion in endometrial carcinoma. *Virchows Arch*. 2017;470:63-68.

102. Guan H, Semaan A, Bandyopadhyay S, Arabi H, Feng J, Fathallah L, et al. Prognosis and reproducibility of new and existing binary grading systems for endometrial carcinoma compared to FIGO grading in hysterectomy specimens. *Int J Gynecol Cancer*. 2011;21:654-660.

103. Gilks CB, Oliva E, Soslow RA. Poor interobserver reproducibility in the diagnosis of high-grade endometrial carcinoma. *Am J Surg Pathol*. 2013;37:874-881.

104. Han G, Sidhu D, Duggan MA, Arseneau J, Cesari M, Clement PB, et al. Reproducibility of histological cell type in high-grade endometrial carcinoma. *Mod Pathol*. 2013;26:1594-1604.

105. Sagae S, Saito T, Satoh M, Ikeda T, Kimura S, Mori M, et al. The reproducibility of a binary tumor grading system for uterine endometrial endometrioid carcinoma, compared with FIGO system and nuclear grading. *Oncology*. 2004;67:344-530.

106. Alkushi A, Abdul-Rahman ZH, Lim P, Schulzer M, Coldman A, Kalloger SE, et al. Description of a novel system for grading of endometrial carcinoma and comparison with existing grading systems. *Am J Surg Pathol*. 2005;29:295-304.

107. Visser NCM, Reijnen C, Massuger L, Nagtegaal ID, Bulten J, Pijnenborg JMA. Accuracy of Endometrial Sampling in Endometrial Carcinoma: A Systematic Review and Meta-analysis. *Obstet Gynecol*. 2017;130:803-813.

108. Banno K, Kisu I, Yanokura M, Tsuji K, Masuda K, Ueki A, et al. Biomarkers in endometrial cancer: Possible clinical applications (Review). *Oncol Lett*. 2012;3:1175-1180.

109. Alkushi A, Kobel M, Kalloger SE, Gilks CB. High-grade endometrial carcinoma: serous and grade 3 endometrioid carcinomas have different immunophenotypes and outcomes. *Int J Gynecol Pathol*. 2010;29:343-350.

110. McConechy MK, Ding J, Cheang MC, Wiegand K, Senz J, Tone A, et al. Use of mutation profiles to refine the classification of endometrial carcinomas. *J Pathol*. 2012;228:20-30.

111. Alvarez T, Miller E, Duska L, Oliva E. Molecular profile of grade 3 endometrioid endometrial carcinoma: is it a type I or type II endometrial carcinoma? *Am J Surg Pathol*. 2012;36:753-761.

112. Santacana M, Maiques O, Valls J, Gatius S, Abo AI, Lopez-Garcia MA, et al. A 9-protein biomarker molecular signature for predicting histologic type in endometrial carcinoma by immunohistochemistry. *Hum Pathol*. 2014;45:2394-2403.

113. Hoang LN, McConechy MK, Kobel M, Han G, Rouzbahman M, Davidson B, et al. Histotype-genotype correlation in 36 high-grade endometrial carcinomas. *Am J Surg Pathol*. 2013;37:1421-1432.

114. Coenegrachts L, Garcia-Dios DA, Depreeuw J, Santacana M, Gatius S, Zikan M, et al. Mutation profile and clinical outcome of mixed endometrioid-serous endometrial carcinomas are different from that of pure endometrioid or serous carcinomas. *Virchows Arch*. 2015;466:415-422.

115. Hoang LN, Lee YS, Karnezis AN, Tessier-Cloutier B, Almandani N, Coatham M, et al. Immunophenotypic features of dedifferentiated endometrial carcinoma - insights from BRG1/INI1-deficient tumours. *Histopathology*. 2016;69:560-569.

116. Ferguson SE, Olshen AB, Viale A, Barakat RR, Boyd J. Stratification of intermediate-risk endometrial cancer patients into groups at high risk or low risk for recurrence based on tumor gene expression profiles. *Clin Cancer Res*. 2005;11:2252-2257.

117. Salvesen HB, Carter SL, Mannelqvist M, Dutt A, Getz G, Stefansson IM, et al. Integrated genomic profiling of endometrial carcinoma associates aggressive tumors with indicators of PI3 kinase activation. *Proc Natl Acad Sci U S A*. 2009;106:4834-9.

118. Wik E, Trovik J, Kusonmano K, Birkeland E, Raeder MB, Pashtan I, et al. Endometrial Carcinoma Recurrence Score (ECARS) validates to identify aggressive disease and associates with markers of epithelial-mesenchymal transition and PI3K alterations. *Gynecol Oncol*. 2014;134:599-606.

119. Stelloo E, Bosse T, Nout RA, MacKay HJ, Church DN, Nijman HW, et al. Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative. *Mod Pathol*. 2015;28:836-844.

120. Stelloo E, Nout RA, Osse EM, Jurgenliemk-Schulz IJ, Jobsen JJ, Lutgens LC, et al. Improved Risk Assessment by Integrating Molecular and Clinicopathological Factors in Early-stage Endometrial Cancer-Combined Analysis of the PORTEC Cohorts. *Clin Cancer Res*. 2016;22:4215-4224.

121. Tang J, Ma W, Luo L. Establishment of the Prognosis Predicting Signature for Endometrial Cancer Patient. *Med Sci Monit*. 2019;25:8248-8259.

122. Zhao D, Ren C, Yao Y, Wang Q, Li F, Li Y, et al. Identifying prognostic biomarkers in endometrial carcinoma based on ceRNA network. *J Cell Biochem*. 2019 Nov 6.

123. Dai D, Thiel KW, Salinas EA, Goodheart MJ, Leslie KK, Gonzalez Bosquet J. Stratification of endometrioid endometrial cancer patients into risk levels using somatic mutations. *Gynecol Oncol*. 2016;142:150-157.

124. Miller MD, Salinas EA, Newtson AM, Sharma D, Keeney ME, Warrier A, et al. An integrated prediction model of recurrence in endometrial endometrioid cancers. *Cancer Manag Res*. 2019;11:5301-5315.

125. Salinas EA, Miller MD, Newtson AM, Sharma D, McDonald ME, Keeney ME, et al. A Prediction Model for Preoperative Risk Assessment in Endometrial Cancer Utilizing Clinical and Molecular Variables. *Int J Mol Sci*. 2019 Mar 9;20(5).

126. Weinberger V, Bednarikova M, Hausnerova J, Ovesna P, Vinklerova P, Minar L, et al. A Novel Approach to Preoperative Risk Stratification in Endometrial Cancer: The Added Value of Immunohistochemical Markers. *Front Oncol*. 2019;9:265.

127. Arend RC, Jones BA, Martinez A, Goodfellow P. Endometrial cancer: Molecular markers and management of advanced stage disease. *Gynecol Oncol*. 2018;150:569-580.

128. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol*. 1983;15:10-7.

129. Cancer Genome Atlas Research N, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. *Nature*. 2013;497:67-73.

130. Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, et al. A clinically applicable molecular-based classification for endometrial cancers. *Br J Cancer*. 2015;113:299-310.

131. Talhouk A, McAlpine JN. New classification of endometrial cancers: the development and potential applications of genomic-based classification in research and clinical care. *Gynecol Oncol Res Pract*. 2016;3:14.

132. Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, et al. Confirmation of ProMisE: A simple, genomics-based clinical classifier for endometrial cancer. *Cancer*. 2017;123:802-813.

133. Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J, et al. Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. *Ann Oncol*. 2018;29:1180-1188.

134. Hoang LN, Kinloch MA, Leo JM, Grondin K, Lee CH, Ewanowich C, et al. Interobserver Agreement in Endometrial Carcinoma Histotype Diagnosis Varies Depending on The Cancer Genome Atlas (TCGA)-based Molecular Subgroup. *Am J Surg Pathol*. 2017;41:245-52.

135. Stelloo E, Nout RA, Naves LC, Ter Haar NT, Creutzberg CL, Smit VT, et al. High concordance of molecular tumor alterations between pre-operative curettage and hysterectomy specimens in patients with endometrial carcinoma. *Gynecol Oncol*. 2014;133:197-204.

136. Talhouk A, Hoang LN, McConechy MK, Nakonechny Q, Leo J, Cheng A, et al. Molecular classification of endometrial carcinoma on diagnostic specimens is highly concordant with final hysterectomy: Earlier prognostic information to guide treatment. *Gynecol Oncol*. 2016;143:46-53.

137. Bendifallah S, Darai E, Ballester M. Predictive Modeling: A New Paradigm for Managing Endometrial Cancer. *Ann Surg Oncol*. 2016;23:975-988.

138. Korkmaz V, Meydanli MM, Yalcin I, Sari ME, Sahin H, Kocaman E, et al. Comparison of three different risk-stratification models for predicting lymph node involvement in endometrioid endometrial cancer clinically confined to the uterus. *J Gynecol Oncol*. 2017;28:e78.

139. Creutzberg CL, van Putten WL, Koper PC, Lybeert ML, Jobsen JJ, Warlam-Rodenhuis CC, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC Study Group. Post Operative Radiation Therapy in Endometrial Carcinoma. *Lancet*. 2000;355:1404-11.

140. Keys HM, Roberts JA, Brunetto VL, Zaino RJ, Spirtos NM, Bloss JD, et al. A phase III trial of surgery with or without adjunctive external pelvic radiation therapy in intermediate risk endometrial adenocarcinoma: a Gynecologic Oncology Group study. *Gynecol Oncol*. 2004;92:744-751.

141. Colombo N, Preti E, Landoni F, Carinelli S, Colombo A, Marini C, et al. Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2013;24 Suppl 6:vi33-8.

142. Bendifallah S, Canlorbe G, Raimond E, Hudry D, Coutant C, Graesslin O, et al. A clue towards improving the European Society of Medical Oncology risk group classification in apparent early stage endometrial cancer? Impact of lymphovascular space invasion. *Br J Cancer*. 2014;110:2640-2646.

143. Vargas R, Rauh-Hain JA, Clemmer J, Clark RM, Goodman A, Growdon WB, et al. Tumor size, depth of invasion, and histologic grade as prognostic factors of lymph node involvement in endometrial cancer: a SEER analysis. *Gynecol Oncol*. 2014;133:216-220.

144. Bendifallah S, Canlorbe G, Collinet P, Arsene E, Huguet F, Coutant C, et al. Just how accurate are the major risk stratification systems for early-stage endometrial cancer? *Br J Cancer*. 2015;112:793-801.

145. Todo Y, Sakuragi N, Nishida R, Yamada T, Ebina Y, Yamamoto R, et al. Combined use of magnetic resonance imaging, CA 125 assay, histologic type, and histologic grade in the prediction of lymph node metastasis in endometrial carcinoma. *Am J Obstet Gynecol*. 2003;188:1265-1272.

146. Lee JY, Jung DC, Park SH, Lim MC, Seo SS, Park SY, et al. Preoperative prediction model of lymph node metastasis in endometrial cancer. *Int J Gynecol Cancer*. 2010;20:1350-1355.

147. Kang S, Kang WD, Chung HH, Jeong DH, Seo SS, Lee JM, et al. Preoperative identification of a low-risk group for lymph node metastasis in endometrial cancer: a Korean gynecologic oncology group study. *J Clin Oncol*. 2012;30:1329-1334.

148. Koskas M, Genin AS, Graesslin O, Barranger E, Haddad B, Darai E, et al. Evaluation of a method of predicting lymph node metastasis in endometrial cancer based on five pre-operative characteristics. *Eur J Obstet Gynecol Reprod Biol*. 2014;172:115-9.

149. Son JH, Kong TW, Kim SH, Paek J, Chang SJ, Lee EJ, et al. Prediction of lymph node metastasis in patients with apparent early endometrial cancer. *Obstet Gynecol Sci*. 2015;58:385-390.

150. Luomaranta A, Leminen A, Loukovaara M. Prediction of lymph node and distant metastasis in patients with endometrial carcinoma: a new model based on demographics, biochemical factors, and tumor histology. *Gynecol Oncol*. 2013;129:28-32.

151. Todo Y, Okamoto K, Hayashi M, Minobe S, Nomura E, Hareyama H, et al. A validation study of a scoring system to estimate the risk of lymph node metastasis for patients with endometrial cancer for tailoring the indication of lymphadenectomy. *Gynecol Oncol*. 2007;104:623-628.

152. Koskas M, Fournier M, Vanderstraeten A, Walker F, Timmerman D, Vergote I, et al. Evaluation of models to predict lymph node metastasis in endometrial cancer: A multicentre study. *Eur J Cancer*. 2016;61:52-60.

153. Installe AJ, Van den Bosch T, De Moor B, Timmerman D. Clinical data miner: an electronic case report form system with integrated data preprocessing and machine-learning libraries supporting clinical diagnostic model research. *JMIR Med Inform*. 2014;2:e28.

154. Heinze G, Dunkler D. Five myths about variable selection. *Transpl Int*. 2017;30:6-10.

155. Wynants L, Bouwmeester W, Moons KG, Moerbeek M, Timmerman D, Van Huffel S, et al. A simulation study of sample size demonstrated the importance of the number of events per variable to develop prediction models in clustered data. *J Clin Epidemiol*. 2015;68:1406-1414.

156. Sullivan TR, Salter AB, Ryan P, Lee KJ. Bias and Precision of the "Multiple Imputation, Then Deletion" Method for Dealing With Missing Outcome Data. *Am J Epidemiol*. 2015;182:528-534.

157. Sauerbrei W, Royston P, Binder H. Selection of important variables and determination of functional form for continuous predictors in multivariable model building. *Stat Med*. 2007;26:5512-5528.

158. Van Houwelingen JC, Le Cessie S. Predictive value of statistical models. *Stat Med*. 1990 Nov;9(11):1303-25.

159. Steyerberg EW. Validation in prediction research: the waste by data splitting. *J Clin Epidemiol*. 2018;103:131-3.

160. Snell KI, Hua H, Debray TP, Ensor J, Look MP, Moons KG, et al. Multivariate meta-analysis of individual participant data helped externally validate the performance and implementation of a prediction model. *J Clin Epidemiol*. 2016;69:40-50.

161. Wynants L, Riley RD, Timmerman D, Van Calster B. Random-effects meta-analysis of the clinical utility of tests and prediction models. *Stat Med*. 2018;37:2034-2052.

162. Trevethan R. Sensitivity, Specificity, and Predictive Values: Foundations, Pliabilities, and Pitfalls in Research and Practice. *Front Public Health*. 2017;5:307.

163. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. *Family medicine*. 2005;37:360-363.

164. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med (Zagreb)*. 2012;22:276-82.

165. Brennan P, Silman A. Statistical methods for assessing observer variability in clinical measures. *BMJ*. 1992;304:1491-1494.

166. Sperandei S. Understanding logistic regression analysis. *Biochem Med (Zagreb)*. 2014;24:12-8.

167. Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ*. 2009;338:b2393.

168. Buuren Sv, Groothuis-Oudshoorn K. mice: Multivariate imputation by chained equations in R. *Journal of statistical software*. 2010:1-68.

169. Lasko TA, Bhagwat JG, Zou KH, Ohno-Machado L. The use of receiver operating characteristic curves in biomedical informatics. *J Biomed Inform*. 2005;38:404-415.

170. Van Calster B, Nieboer D, Vergouwe Y, De Cock B, Pencina MJ, Steyerberg EW. A calibration hierarchy for risk models was defined: from utopia to empirical data. *J Clin Epidemiol*. 2016;74:167-176.

171. Fitzgerald M, Saville BR, Lewis RJ. Decision curve analysis. *JAMA*. 2015;313:409-10.

172. Vickers AJ, Van Calster B, Steyerberg EW. Net benefit approaches to the evaluation of prediction models, molecular markers, and diagnostic tests. *BMJ*. 2016;352:i6.

173. George B, Seals S, Aban I. Survival analysis and regression models. *J Nucl Cardiol*. 2014;21:686-694.

174. Rich JT, Neely JG, Paniello RC, Voelker CC, Nussenbaum B, Wang EW. A practical guide to understanding Kaplan-Meier curves. *Otolaryngol Head Neck Surg*. 2010;143:331-336.

175. Ytre-Hauge S, Husby JA, Magnussen IJ, Werner HM, Salvesen OO, Bjorge L, et al. Preoperative tumor size at MRI predicts deep myometrial invasion, lymph node metastases, and patient outcome in endometrial carcinomas. *Int J Gynecol Cancer*. 2015;25:459-466.

176. Green RW, Epstein E. Can dynamic contrast-enhanced ultrasound (DCE-US) improve diagnostic performance in endometrial cancer staging? A proof of concept. *Ultrasound Obstet Gynecol*. 2019 Oct 24.

177. Green RW, Valentin L, Alcazar JL, Chiappa V, Erdodi B, Franchi D, et al. Endometrial cancer off-line staging using two-dimensional transvaginal ultrasound and three-dimensional volume contrast imaging: Intermethod agreement, interrater reliability and diagnostic accuracy. *Gynecol Oncol*. 2018;150:438-445.

178. Eriksson LS, Lindqvist PG, Floter Radestad A, Dueholm M, Fischerova D, Franchi D, et al. Transvaginal ultrasound assessment of myometrial and cervical stromal invasion in women with endometrial cancer: interobserver reproducibility among ultrasound experts and gynecologists. *Ultrasound Obstet Gynecol*. 2015;45:476-482.

179. Geppert B, Lonnerfors C, Bollino M, Persson J. Sentinel lymph node biopsy in endometrial cancer-Feasibility, safety and lymphatic complications. *Gynecol Oncol*. 2018;148:491-498.

180. Hareyama H, Ito K, Hada K, Uchida A, Hayakashi Y, Hirayama E, et al. Reduction/prevention of lower extremity lymphedema after pelvic and para-aortic lymphadenectomy for patients with gynecologic malignancies. *Ann Surg Oncol*. 2012;19:268-273.

181. How JA, O'Farrell P, Amajoud Z, Lau S, Salvador S, How E, et al. Sentinel lymph node mapping in endometrial cancer: a systematic review and meta-analysis. *Minerva Ginecol*. 2018;70:194-214.

182. Bodurtha Smith AJ, Fader AN, Tanner EJ. Sentinel lymph node assessment in endometrial cancer: a systematic review and meta-analysis. *Am J Obstet Gynecol*. 2017;216:459-76 e10.

183. Katz MH. Multivariable analysis: a primer for readers of medical research. *Ann Intern Med*. 2003;138:644-650.