From the Department of Women's and Children's Health
Division of Obstetrics and Gynecology
Karolinska Institutet, Stockholm, Sweden

ULTRASOUND ASSESSMENT AND RISK PREDICTION IN WOMEN WITH ENDOMETRIAL CANCER

Linda Eriksson

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Ultrasound assessment and risk prediction in women with endometrial cancer

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By

Linda Eriksson

Principal Supervisor:
Associate Professor Elisabeth Epstein
Karolinska Institutet
Department of Clinical Science and Education
Division of Obstetrics and Gynecology

Co-supervisor(s):
Associate Professor Angelique Flöter Rådestad
Karolinska Institutet
Department of Women's and Children's Health
Division of Obstetrics and Gynecology

Associate Professor Pelle Lindqvist
Karolinska Institutet
Department of Clinical Science and Education
Division of Obstetrics and Gynecology

Opponent:
MD, PhD Eva Dreisler
University of Copenhagen
Department of Gynecology

Examination Board:
Professor Karin Sundfeldt
Gothenburg University
Department of Obstetrics and Gynecology
Institute of Clinical Sciences, Sahlgrenska Academy

Associate Professor Torkel Brismar
Karolinska Institutet
Department of Clinical Science, Intervention and Technology (CLINTEC)
Division of Radiology

Associate Professor Anders Nässman
Karolinska Institutet
Department of Oncology-Pathology
"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


*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


*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


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

Manuscript. Accepted for publication in *Ultrasound Obstet Gynecol*


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

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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
<table>
<thead>
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<tr>
<td>POLE EDM</td>
<td>Polymerase-ε Exonuclease Domain Mutations</td>
</tr>
<tr>
<td>PORTEC</td>
<td>Post Operative Radiation Therapy in Endometrial Carcinoma</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive Predictive Value</td>
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<tr>
<td>ProMisE</td>
<td>Proactive Molecular Risk Classifier for Endometrial Cancer</td>
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<tr>
<td>PTEN</td>
<td>Phosphatase and Tensin Homologue</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
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<tr>
<td>ROC</td>
<td>Receiver Operating Characteristic</td>
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<tr>
<td>SEPAL</td>
<td>Survival Effect of Para-Aortic Lymphadenectomy in Endometrial Cancer</td>
</tr>
<tr>
<td>TCGA</td>
<td>The Cancer Genome Atlas</td>
</tr>
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<td>US</td>
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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\(^16-8,11\).

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\textsuperscript{31} and the TNM classification\textsuperscript{32} are the staging systems most in use\textsuperscript{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\textsuperscript{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\textsuperscript{34}.

A 2017 Cochrane review\textsuperscript{35} based on two randomized controlled trials (RCT); Benedetti-Panici et al\textsuperscript{36} and the ASTEC trial\textsuperscript{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\textsuperscript{38} for a short follow-up period (median 37 months, with 35.7% of surviving patients followed-up for less than 3 years)\textsuperscript{37}, selective rather than systematic lymphadenectomy (removal of 9 or fewer lymph nodes in 35% of women in the lymphadenectomy group)\textsuperscript{37}, for not including para-aortic lymphadenectomy\textsuperscript{36,37} and for not assessing risk of recurrence\textsuperscript{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\textsuperscript{38}.

No RCT evidence shows the effect of lymphadenectomy in women at high risk of recurrence or higher-stage disease\textsuperscript{35}. 

2
### Table 1. Staging of endometrial cancer according to FIGO over time

| 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 |  |
| 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\(^\text{27}\).

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 true-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.

Extraterine tumor extension should be described, using both transvaginal- and transabdominal ultrasound\(^\text{39-41}\).
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 permission.

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), tumor/uterine anteroposterior (AP) diameter ratio, (Karlsson's ratio), endometrial thickness, tumor/uterine volume ratio, minimal tumor-free margin/uterine AP diameter ratio and the shortest distance to serosa (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 (Figure 1).
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\textsuperscript{44,56} and 29%—93% vs. 85%—99%, respectively, for cervical stromal invasion\textsuperscript{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\textsuperscript{63}.

The adnexal region should be assessed, to rule out ovarian spread or synchronous ovarian cancer, reported to occur in 5% of cases\textsuperscript{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%)\textsuperscript{58}.

Figure 2. Ultrasound tumor extension: a) myometrial invasion < 50%, b) myometrial invasion ≥ 50%, c) no cervical stromal invasion, d) cervical stromal invasion.
Table 2. Subjective assessment of deep myometrial invasion (≥ 50%) and cervical stromal invasion by transvaginal ultrasound

<table>
<thead>
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<th>N=</th>
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<th>Cervical stromal invasion</th>
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<tr>
<td></td>
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<td>De Smet et al.</td>
<td>2006</td>
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<tr>
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<td>2019</td>
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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\(^4\)

**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\(^4\)
Figure 5. Endometrial midline: a) linear, b) non-linear, c) irregular and d) not defined. Reprinted with permission⁴⁰

Figure 6. Bright edge. Reprinted with permission⁴⁰

Figure 7. Endometrial-myometrial junction: a) regular, b) irregular, c) interrupted and d) not defined. Reprinted with permission⁴⁰
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\(^{40}\)](image)

![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\(^{40}\)](image)
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, \((\text{AP diameter} \times \text{length} \times \text{width}) / 2\)^{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.](image)

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-analyses 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**

<table>
<thead>
<tr>
<th>Year</th>
<th>N=</th>
<th>Ultrasound Sensitivity (%)</th>
<th>Ultrasound Specificity (%)</th>
<th>MRI Sensitivity (%)</th>
<th>MRI Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cicinelli et al</td>
<td>2008</td>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Savelli et al</td>
<td>2008</td>
<td>74</td>
<td>84</td>
<td>83</td>
<td>84</td>
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<tr>
<td>Ozdemir et al</td>
<td>2009</td>
<td>64</td>
<td>85</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>Celik et al</td>
<td>2010</td>
<td>64</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Antonsen et al</td>
<td>2013</td>
<td>123</td>
<td>69</td>
<td>74</td>
<td>89</td>
</tr>
<tr>
<td>Ortoft et al</td>
<td>2013</td>
<td>156</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Christensen et al</td>
<td>2016</td>
<td>110</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**b. Cervical stromal invasion**

<table>
<thead>
<tr>
<th>Year</th>
<th>N=</th>
<th>Ultrasound Sensitivity (%)</th>
<th>Ultrasound Specificity (%)</th>
<th>MRI Sensitivity (%)</th>
<th>MRI Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cicinelli et al</td>
<td>2008</td>
<td>100</td>
<td>53</td>
<td>82</td>
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<tr>
<td>Savelli et al</td>
<td>2008</td>
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<td>93</td>
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<tr>
<td>Ozdemir et al</td>
<td>2009</td>
<td>64</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Celik et al</td>
<td>2010</td>
<td>64</td>
<td>88</td>
<td>92</td>
<td>88</td>
</tr>
<tr>
<td>Antonsen et al</td>
<td>2013</td>
<td>123</td>
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<td>94</td>
<td>27</td>
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<tr>
<td>Ortoft et al</td>
<td>2013</td>
<td>156</td>
<td>38</td>
<td>89</td>
<td>54</td>
</tr>
<tr>
<td>Christensen et al</td>
<td>2016</td>
<td>110</td>
<td>39</td>
<td>88</td>
<td>56</td>
</tr>
</tbody>
</table>

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.\(^7\)

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.

### 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: \( \leq 5\% \) 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. \( \geq 50\% \)), the presence of cervical stromal invasion (no vs. yes) and extraterine 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 \cite{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 \cite{85-90,93} and an increased risk of recurrence \cite{94}.

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

### 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 \cite{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) \cite{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) \cite{99-101}

Tumor histotype and grade have limited interobserver reproducibility (κ 0.62—0.87 vs. 0.35—0.65, respectively) \cite{78,102}, especially in high-grade tumors, as defined by grade 3 endometrioid cancer or non-endometrioid cancer \cite{103,104}. Binary grading systems have been
associated with higher interobserver reproducibility than the three-tire FIGO grading system. 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.

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.

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.

### 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. 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, to detect prognostic subgroups, in risk prediction models and in individualized therapy.

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. Molecular data have supported this dichotomous classification, with genetic alterations in PTEN, KRAS2, CTNNB1 and PIK3CA and LMH1 promoter hypermethylation in type I cancer and mutations in p53 and HER-2/neu in type II cancer. However, considerable molecular heterogeneity exists, with expression of p53 mutations in 25% of endometrioid grade 3 cancer. Also, its prognostic value is limited, as 20% of type I cancer relapse, whereas 50% of type II cancers do not.

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). 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. 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\(^{140}\) (Gynecologic 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\(^{25, 53, 61, 145-149}\) 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

<table>
<thead>
<tr>
<th>Classification</th>
<th>Year</th>
<th>N</th>
<th>Variables</th>
</tr>
</thead>
</table>
| Mayo criteria &nbsp;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 &nbsp;⁹⁹⁹            | 2000 | 715 | Age  
Histotype (endometrioid vs. non-endometrioid)  
FIGO grade (1 vs. 2 vs. 3)  
Myometrial invasion (< 50% vs. ≥ 50%) |
| GOG-99 &nbsp;⁹⁹⁹            | 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 &nbsp;⁹⁶               | 2010 | 671 | Histologic type (endometrioid vs. non-endometrioid)  
FIGO grade (1 vs. 2 vs. 3)  
FIGO stage  
LVSI |
| ESMO &nbsp;⁹⁴                | 2013 | —   | Histologic type (endometrioid vs. non-endometrioid)  
FIGO grade (1 vs. 2 vs. 3)  
Myometrial invasion (< 50% vs. ≥ 50%) |
| ESMO modified &nbsp;⁹⁴       | 2014 | 496 | Histologic type (endometrioid vs. non-endometrioid)  
FIGO grade (1 vs. 2 vs. 3)  
Myometrial invasion (< 50% vs. ≥ 50%)  
LVSI |
| Mayo-modified criteria &nbsp;⁹⁴ | 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

<table>
<thead>
<tr>
<th>Year</th>
<th>N</th>
<th>Cohort</th>
<th>Histotype</th>
<th>Staging</th>
<th>Imaging</th>
<th>Predictors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Todo</td>
<td>2003</td>
<td>214 Retrospective Multicenter Treatment between 1993—2000</td>
<td>Endometrioid and non-endometrioid</td>
<td>FIGO 1988 Stage I—IV</td>
<td>MRI</td>
<td>Pelvic lymph node metastases: Serous histotype (yes vs. no). Volume index (&lt; 25 vs. ≥ 25). Grade (1—2 vs. 3). CA 125 (&lt; 70 vs. ≥ 70 if age &lt;50 years and &lt; 28 vs. ≥ 28 if age ≥ 50 years). Para-aortic lymph node metastases: Volume index (&lt; 40 vs. ≥ 40). CA 125 (&lt; 70 vs. ≥ 70 if age &lt; 50 years and &lt; 28 vs. ≥ 28 if age ≥ 50 years).</td>
</tr>
<tr>
<td>Lee</td>
<td>2010</td>
<td>110 Retrospective Presumed one center Treatment between 2001—2008</td>
<td>Endometrioid and non-endometrioid</td>
<td>FIGO 1988 Stage I—IV</td>
<td>MRI</td>
<td>CA 125 (&lt; 70 vs. ≥ 70). Any MI (yes/no). Grade (1 vs. 2—3). Extension beyond uterus (yes vs. no).</td>
</tr>
<tr>
<td>Kang</td>
<td>2012</td>
<td>360 Retrospective Multicenter Treatment between 2002—2008</td>
<td>Endometrioid</td>
<td>FIGO 1988 Stage I—IV</td>
<td>MRI</td>
<td>CA 125 (&lt; 35 vs. ≥ 35). MI (&lt; 50% vs. ≥ 50%). Extension beyond corpus (yes vs. no). Enlarged lymph nodes (yes vs. no).</td>
</tr>
<tr>
<td>Koskas</td>
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<td>181 Retrospective Multicenter Treatment between 2000—2010</td>
<td>Endometrioid and non-endometrioid</td>
<td>Presumed FIGO 2009 Tentative stage I—II</td>
<td>MRI</td>
<td>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 &lt; 50% vs. MI ≥ 50% vs. CSI).</td>
</tr>
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<td>Son</td>
<td>2015</td>
<td>142 Retrospective One center Treatment between 2000—2013</td>
<td>Endometrioid grade 1—2</td>
<td>Presumed FIGO 2009 Tentative Stage IA</td>
<td>MRI</td>
<td>Grade 1 and CA 125 &lt; 35 vs. grade 2 or CA 125 ≥ 35</td>
</tr>
<tr>
<td>Lee</td>
<td>2016</td>
<td>172 Retrospective Presumed one center Treatment between 2000—2013</td>
<td>Endometrioid and non-endometrioid</td>
<td>FIGO 2009 Stage I—IV</td>
<td>TVS</td>
<td>MI (&lt; 50% vs. ≥ 50%). Grade (1 vs. 2—3). CA 125 (&lt; 35 vs. ≥ 35).</td>
</tr>
</tbody>
</table>

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

* The nomogram by Bendifallah et al14 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\textsuperscript{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\textsuperscript{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 on women with endometrial cancer, examined preoperatively with ultrasound according to the IETA study protocol (Figure 12).

![Flowchart of the study populations](image)

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, performed at another hospital or ≥ 120 days after ultrasound examination, n = 118.

Diagnoses other than endometrial cancer, n = 25.

Missing ultrasound data due to incorrect fillout of the study protocol, n = 26.

Duplicate entries, n = 5.

Error in the identification key, n = 2.

Ultrasound data was obtained by experienced ultrasound examiners, using high-end ultrasound equipment and following the IETA examination technique and terminology, previously described. 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 \(((\text{AP-diameter} \times \text{length} \times \text{width}) / 2)\) (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\textsuperscript{31} and grade was classified according to FIGO\textsuperscript{79}.

Data on demography and ultrasound variables were entered into an internet-based data capture software, Clinical Data Miner (https://cdm.esat.kuleuven.be)\textsuperscript{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

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

*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 video clips 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 video clips 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), video clip 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. 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. 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 unmeasurable 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\textsuperscript{132} MMR-D: mismatch repair proteins deficiency; POLE EDM: polymerase-ε exonuclease domain mutations; p53 wt: 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\textsuperscript{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

<table>
<thead>
<tr>
<th>Type</th>
<th>Risk group</th>
<th>Description*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative†</td>
<td>Low</td>
<td>Endometrioid grade 1—2 with MI &lt; 50%</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>Endometrioid grade 1—2 with MI ≥ 50%</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>Endometrioid grade 3 with MI ≥ 50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-endometrioid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postoperative‡</td>
<td>Low</td>
<td>Stage I endometrioid, grade 1—2, &lt; 50% MI, LVSI neg</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>Stage I endometrioid, grade 1—2, ≥ 50% MI, LVSI neg</td>
</tr>
<tr>
<td></td>
<td>High intermediate</td>
<td>Stage I endometrioid, grade 3, &lt; 50% MI, LVSI neg or pos</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>Stage I endometrioid, grade 1—2, LVSI pos, MI &lt; or ≥ 50%</td>
</tr>
<tr>
<td></td>
<td>Advanced</td>
<td>Stage III without residual disease</td>
</tr>
<tr>
<td></td>
<td>Metastatic</td>
<td>Stage IVB</td>
</tr>
</tbody>
</table>

*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). McNemar's 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).

\[
\begin{array}{ccc}
\text{Condition present} & \text{Condition absent} \\
\hline
\text{Test positive} & \text{Test negative} & \\
\text{True positive} & \text{False positive/Type I error} & \\
\text{False negative/Type II error} & \text{True negative} & \\
\end{array}
\]

\[
\begin{array}{ccc}
\frac{a}{a+b} &= & \text{PPV} \\
\frac{d}{c+d} &= & \text{NPV} \\
\frac{a}{a+c} &= & \text{sensitivity} \\
\frac{d}{b+d} &= & \text{specificity} \\
\frac{a+d}{a+b+c+d} &= & \text{accuracy}
\end{array}
\]

**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" ($\leq 0.20$), "fair" (0.21—0.40), "moderate" (0.41—0.60), "good" (0.61—0.80) and "very good" (0.81—1.00)\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{167, 168}. 

30
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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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 (\( \geq 50\% \)) and cervical stromal invasion by ultrasound experts and gynecologists

<table>
<thead>
<tr>
<th></th>
<th>Ultrasound experts</th>
<th>Gynecologists</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value 95% CI</td>
<td>Value 95% CI</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>73 66—79</td>
<td>73 66—79</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>69 63—74</td>
<td>70 65—75</td>
</tr>
<tr>
<td>Kappa(^\dagger)</td>
<td>0.52 0.48—0.57</td>
<td>0.48 0.44—0.53</td>
</tr>
<tr>
<td>Certainty (VAS)</td>
<td>74 63—84</td>
<td>56 48—65</td>
</tr>
<tr>
<td>Quality (VAS)</td>
<td>64 52—77</td>
<td>56 50—63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Ultrasound experts</th>
<th>Gynecologists</th>
<th>( p^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>57 45—68</td>
<td>42 31—53</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>87 83—90</td>
<td>83 78—86</td>
<td>0.02</td>
</tr>
<tr>
<td>Kappa(^\dagger)</td>
<td>0.58 0.53—0.62</td>
<td>0.45 0.40—0.49</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Certainty (VAS)</td>
<td>77 65—89</td>
<td>58 48—68</td>
<td>0.01</td>
</tr>
<tr>
<td>Quality (VAS)</td>
<td>71 57—85</td>
<td>59 53—64</td>
<td>0.07</td>
</tr>
</tbody>
</table>

*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 \( \dagger \) 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

<table>
<thead>
<tr>
<th>Kappa</th>
<th>Deep myometrial invasion n (%)</th>
<th>Cervical stromal invasion n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ultrasound experts</td>
<td>Gynecologists</td>
</tr>
<tr>
<td>Poor (&lt; 0.20)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fair (0.21—0.40)</td>
<td>7 (19)</td>
<td>7 (19)</td>
</tr>
<tr>
<td>Moderate (0.41—0.60)</td>
<td>27 (47)</td>
<td>21 (58)</td>
</tr>
<tr>
<td>Good (0.61—0.80)</td>
<td>11 (31)</td>
<td>8 (22)</td>
</tr>
<tr>
<td>Very good (0.81—1.00)</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

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 (%)

<table>
<thead>
<tr>
<th></th>
<th>No MELF</th>
<th>MELF</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>71</td>
<td>71</td>
<td>0.95</td>
</tr>
<tr>
<td>Specificity</td>
<td>79</td>
<td>76</td>
<td>0.41</td>
</tr>
<tr>
<td>Accuracy</td>
<td>77</td>
<td>73</td>
<td>0.28</td>
</tr>
<tr>
<td>PPV</td>
<td>57</td>
<td>76</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>NPV</td>
<td>87</td>
<td>70</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

*χ² test

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

<table>
<thead>
<tr>
<th></th>
<th>Univariable analysis</th>
<th>Multivariable analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95 % CI</td>
<td>p</td>
</tr>
<tr>
<td>MELF present</td>
<td>2.6 1.9—3.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Grade 1</td>
<td>Ref</td>
<td>—</td>
</tr>
<tr>
<td>Grade 2</td>
<td>2.6 1.9—3.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Grade 3</td>
<td>4.7 3.1—7.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>MI ≥ 50%</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CSI</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MI &lt; 50%, no CSI</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MI ≥ 50%, no CSI</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CSI, MI &lt; 50% / ≥ 50%</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

b. Lymph node metastases

<table>
<thead>
<tr>
<th></th>
<th>Univariable analysis</th>
<th>Multivariable analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95 % CI</td>
<td>p</td>
</tr>
<tr>
<td>MELF present</td>
<td>2.2 1.2—4.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Grade 1</td>
<td>Ref</td>
<td>—</td>
</tr>
<tr>
<td>Grade 2</td>
<td>1.4 0.6—3.3</td>
<td>0.51</td>
</tr>
<tr>
<td>Grade 3</td>
<td>2.4 1.0—5.9</td>
<td>0.06</td>
</tr>
<tr>
<td>MI ≥ 50%</td>
<td>8.0 3.8—16.8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>CSI</td>
<td>4.0 2.1—7.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>MI &lt; 50%, no CSI</td>
<td>Ref</td>
<td>—</td>
</tr>
<tr>
<td>MI ≥ 50%, no CSI</td>
<td>5.6</td>
<td>2.5—12.2</td>
</tr>
<tr>
<td>CSI, MI &lt;50% / ≥ 50%</td>
<td>10.8</td>
<td>4.5—25.8</td>
</tr>
</tbody>
</table>

*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)

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

Exclusion of predictors was based on $\alpha = 0.2$, 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).

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

\[X\beta =
\begin{align*}
-3.962 \\
+ 0.134 \times \text{(age in years / 10)} \\
+ 0.072 \times \text{(duration of abnormal bleeding in months / 3)} \\
+ 0.607 \times (1 \text{ if non-endometrioid cancer on endometrial biopsy, 0 otherwise}) \\
+ 0.592 \times (1 \text{ if clinical suspicion of cancer}, 0 \text{ otherwise}) \\
+ 0.502 \times (1 \text{ if MI} \geq 50\% \text{ without CSI or extraterine spread on ultrasound, 0 otherwise}) \\
+ 0.512 \times (1 \text{ if CSI without extraterine spread on ultrasound, 0 otherwise}) \\
+ 1.533 \times (1 \text{ if extraterine spread on ultrasound, 0 otherwise}) \\
+ 0.359 \times (\text{AP tumor/uterine diameter ratio / 0.25}) \\
+ 1.046 \times (1 \text{ if undefined tumor with unmeasurable endometrium, 0 otherwise})
\end{align*}
\]

**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)

<table>
<thead>
<tr>
<th>Model risk threshold</th>
<th>Test positive</th>
<th>95 % CI</th>
<th>Sensitivity</th>
<th>95 % CI</th>
<th>Specificity</th>
<th>95 % CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>97</td>
<td>96—98</td>
<td>99</td>
<td>94—100</td>
<td>3</td>
<td>2—4</td>
</tr>
<tr>
<td>0.05</td>
<td>90</td>
<td>88—92</td>
<td>98</td>
<td>92—99</td>
<td>11</td>
<td>9—14</td>
</tr>
<tr>
<td>0.1</td>
<td>58</td>
<td>54—62</td>
<td>83</td>
<td>76—89</td>
<td>47</td>
<td>41—52</td>
</tr>
<tr>
<td>0.15</td>
<td>39</td>
<td>35—43</td>
<td>67</td>
<td>58—75</td>
<td>66</td>
<td>61—71</td>
</tr>
<tr>
<td>0.2</td>
<td>24</td>
<td>21—28</td>
<td>48</td>
<td>39—56</td>
<td>80</td>
<td>75—85</td>
</tr>
<tr>
<td>0.3</td>
<td>9</td>
<td>7—12</td>
<td>25</td>
<td>18—33</td>
<td>94</td>
<td>90—96</td>
</tr>
<tr>
<td>0.4</td>
<td>4</td>
<td>3—6</td>
<td>13</td>
<td>8—20</td>
<td>97</td>
<td>95—99</td>
</tr>
<tr>
<td>0.5</td>
<td>2</td>
<td>1—3</td>
<td>5</td>
<td>3—11</td>
<td>99</td>
<td>97—100</td>
</tr>
</tbody>
</table>

Comparison of the model, biopsy alone and combined biopsy and ultrasound

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.196*</td>
<td></td>
<td>25</td>
<td>22—29</td>
<td>50</td>
<td>41—58</td>
<td>80</td>
</tr>
<tr>
<td>Biopsy†</td>
<td></td>
<td>25</td>
<td>21—29</td>
<td>35</td>
<td>27—44</td>
<td>77</td>
</tr>
<tr>
<td>0.113*</td>
<td></td>
<td>52</td>
<td>48—56</td>
<td>80</td>
<td>71—86</td>
<td>53</td>
</tr>
<tr>
<td>Biopsy + ultrasound‡</td>
<td>52</td>
<td>47—57</td>
<td>75</td>
<td>66—81</td>
<td>52</td>
<td>46—59</td>
</tr>
</tbody>
</table>

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 \( \geq 2 \) 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)

<table>
<thead>
<tr>
<th>Number at risk</th>
<th>Time (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>MMR-D</td>
<td>118</td>
</tr>
<tr>
<td>POLE EDM</td>
<td>26</td>
</tr>
<tr>
<td>p53 wt</td>
<td>151</td>
</tr>
<tr>
<td>p53 abn</td>
<td>44</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Demographic variables</th>
<th>Histotype and grade</th>
<th>ProMisE model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>HR 95% CI p*</td>
<td>HR 95% CI p*</td>
</tr>
<tr>
<td>&lt; 65</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>≥ 65</td>
<td>4.0 1.7—9.5</td>
<td>4.4 2.0—9.8</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>&lt; 88</td>
<td>2.6 1.2—5.6</td>
<td>2.5 1.2—5.4</td>
</tr>
<tr>
<td>≥ 88</td>
<td>Ref</td>
<td>Ref</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histopathological variables</th>
<th>Histotype and grade</th>
<th>ProMisE model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrioid grade 1—2</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Endometrioid grade 3</td>
<td>2.0 0.8—5.0</td>
<td>2.6 1.1—6.0</td>
</tr>
<tr>
<td>Non-endometrioid</td>
<td>1.9 0.7—4.8</td>
<td>4.4 2.3—8.2</td>
</tr>
<tr>
<td>Other†</td>
<td>0.8 0.2—3.5</td>
<td>0.8 0.2—3.4</td>
</tr>
<tr>
<td>p53 wt</td>
<td>Ref</td>
<td>—</td>
</tr>
<tr>
<td>MMR-D</td>
<td>1.1 0.5—2.4</td>
<td>—</td>
</tr>
<tr>
<td>POLE EDM</td>
<td>1.0 0.2—5.1</td>
<td>—</td>
</tr>
<tr>
<td>p53 abn</td>
<td>3.9 1.3—11.1</td>
<td>—</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ultrasound variables</th>
<th>Histotype and grade</th>
<th>ProMisE model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor extension</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>&lt;2</td>
<td>4.7 1.8—12.4</td>
<td>3.9 1.6—9.7</td>
</tr>
<tr>
<td>Tumor not defined</td>
<td>5.3 1.02—27.2</td>
<td>4.2 1.1—16.7</td>
</tr>
<tr>
<td>Endometrial-myometrial junction†</td>
<td>0.30</td>
<td>—</td>
</tr>
<tr>
<td>Regular</td>
<td>Ref</td>
<td>—</td>
</tr>
<tr>
<td>Irregular/interrupted/undefined</td>
<td>2.0 0.6—7.4</td>
<td>—</td>
</tr>
<tr>
<td>Endometrial morphology‡</td>
<td>0.30</td>
<td>—</td>
</tr>
<tr>
<td>Uniform</td>
<td>Ref</td>
<td>—</td>
</tr>
<tr>
<td>Non-uniform</td>
<td>1.3 0.7—2.4</td>
<td>—</td>
</tr>
<tr>
<td>Color score‡</td>
<td>0.07</td>
<td>—</td>
</tr>
<tr>
<td>1—2</td>
<td>Ref</td>
<td>—</td>
</tr>
<tr>
<td>3—4</td>
<td>0.4 0.2—1.1</td>
<td>—</td>
</tr>
<tr>
<td>Vascular pattern‡</td>
<td>0.60</td>
<td>—</td>
</tr>
<tr>
<td>Other</td>
<td>Ref</td>
<td>—</td>
</tr>
<tr>
<td>Multiple multifocal</td>
<td>1.2 0.5—3.0</td>
<td>—</td>
</tr>
</tbody>
</table>

* 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).

\[\text{ESMO pre (preoperative ESMO classification): } \text{AUC} 0.74 \ (95\% \ CI 0.67—0.82)\]
\[\text{ESMO post (postoperative ESMO classification): } \text{AUC} 0.79 \ (95\% \ CI 0.72—0.86)\]
\[\text{Histotype and grade model: } \text{AUC} 0.88 \ (95\% \ CI 0.83—0.92)\]
\[\text{ProMisE model: } \text{AUC} 0.89 \ (95\% \ CI 0.85—0.93)\]
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 (≥1B) 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 metastases5, 10, 13-16, 18-22, 24, 25, recurrence and survival7, 8 and presence of cervical stromal invasion confers a higher stage, associated with worse survival7. Because of the increased risk of adverse outcome, preoperative assessment of deep myometrial invasion and cervical stromal invasion is included in preoperative risk classification27, 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 techniques54-56. 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 guidelines27. 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\textsuperscript{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\textsuperscript{63}.

According to \textbf{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 \textbf{Study IV}, deep myometrial invasion was significantly more common in tumors with AP diameter $\geq$ 2 cm, verifying that the risk of deep myometrial invasion increases with tumor size\textsuperscript{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\textsuperscript{176} and hence has the possibility to improve preoperative risk classification.

\section*{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\%\textsuperscript{58}, MRI: 95\% vs. 59\%\textsuperscript{76}, PET/CT: 93\% vs. 74\%\textsuperscript{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\textsuperscript{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\textsuperscript{27}. In \textbf{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\textsuperscript{27}.

Though grade is an established risk factor for lymph node metastases\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{107}, ProMisE is associated with a high concordance between preoperative biopsy and hysterectomy specimen\textsuperscript{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 $\geq 65$ years, waist circumference $\geq 88$ cm), sonographic (tumor extension and ultrasound tumor AP diameter $\geq 2$ 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 $\geq 88$ 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\textsuperscript{133,135,136}.

5.4 YES, SIZE MATTERS

Tumor size on MRI\textsuperscript{12,175}, hysterectomy specimen\textsuperscript{5, 6, 10, 11, 13, 15, 17-19, 23, 24} and ultrasound\textsuperscript{66, 69}, is predictive of deep myometrial invasion\textsuperscript{69,175}, high-risk disease\textsuperscript{66}, lymph node metastases\textsuperscript{5, 6, 10-13, 15, 17-19, 23, 24, 69, 175} and recurrence and survival\textsuperscript{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\textsuperscript{27}, the GOG-99 criteria\textsuperscript{140}, the SEPAL criteria\textsuperscript{38} or the PORTEC 1 criteria\textsuperscript{139}, as opposed to the Mayo criteria\textsuperscript{6} and the Mayo-modified criteria\textsuperscript{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 shows that 2D ultrasound predicts deep myometrial invasion and cervical stromal invasion with higher interobserver reproducibility than 3D volume contrast imaging (deep myometrial invasion: \( \kappa \) 0.41 vs. \( \kappa \) 0.31 and cervical stromal invasion: \( \kappa \) 0.55 vs. \( \kappa \) 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)\textsuperscript{181} vs. 96% (95% CI 91—98)\textsuperscript{182} and the NPV 100% (95% CI 99—100)\textsuperscript{181} vs. 100% (95% CI not stated)\textsuperscript{182}. However, bilateral pelvic sentinel node detection rate was only 61% (95% CI 56—66)\textsuperscript{181} vs. 50% (95% CI 44—56)\textsuperscript{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 extraterine 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 video clips 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 video clips 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 too old or too 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\textsuperscript{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\textsuperscript{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 cohort133.
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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