RISK PROFILING OF ENDOMETRIAL CANCER

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ACADEMIC DISSERTATION

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Helsinki 2019
The Faculty of Medicine uses the Urkund system (plagiarism recognition) to examine all doctoral dissertations.
To my family
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:


Risk profiling is crucial in preparing both operative and adjuvant treatment of patients with endometrial cancer (EC). The rationale of lymphadenectomy manifests in prognostication and proper triage of patients for adjuvant therapy, allowing the omission of lymphadenectomy and/or adjuvant treatments in patients who are at low risk for advanced disease. Extensive surgery exposes the patient to cumbersome complications. Still, even the early stage low-risk cancers sometimes relapse.

This thesis consists of four cohort studies that were conducted to evaluate the reliability of methods that are presently accessible for the risk profiling of endometrial cancer treatment and to augment contemporary ways that might be clinically usable. Cohort studies were based on a sample of 1166 women who were surgically treated for endometrial cancer at the Department of Obstetrics and Gynecology, Helsinki University Hospital, between January 2007 and December 2013.

In the first study, including the whole cohort of 1166 women, previously recognized risk factors for advanced stage and poor outcome were used to create a calculatory score to predict lymph node and distant metastasis in EC. The association of stage IIIC–IV disease with demographic factors (age, body mass index), biochemical factors (complete blood count, serum CA-125), preoperative histology and tumor size was examined in unadjusted analyses. Logistic regression analysis was used for the identification of variables that independently predict an advanced disease. Statistically significant odds ratios were rounded to the nearest integer and divided by a factor of 2, therefore giving thrombocytosis 1 point, G3/nonendometrioid histology 2 points, tumor diameter > 3 cm 2 points and CA-125 > 35U/ml 3 points. Depending on the number of risk factors of an individual patient, the score ranged from 0 to 8 points. Using 1 as the cut-point for positive and negative test results, the model predicted stage IIIC–IV carcinomas with a sensitivity of 100%, specificity of 38.0%, PPV of 17.1% and NPV of 100%. With the same cut-off, the corresponding values were 100%, 34.7%, 16.5% and 100% in predicting stage IIIC carcinomas in a subgroup that underwent lymphadenectomy and had a stage I–IIIC disease, suggesting that the model predicts lymph node metastases (LNM) in addition to distant metastases.

In the second study on the 1052 stage I–III endometrioid endometrial cancer (EEC) woman cohort, the risk scoring system developed in study I was evaluated by comparing its performance characteristics with two other risk models in predicting lymphatic dissemination in EC. The models had similar accuracies in predicting lymphatic dissemination. The lymphadenectomy rate was lowest for a
Milwaukee model. Survival analyses suggest that variables included in the models predict patient outcome independently of tumor stage.

The third study was conducted to investigate the correlation of predictors of advanced disease and/or poor outcome with the manifestation of tumor relapses in different anatomical sites in patients with stage I–II EEC. A relapse was diagnosed in 98 patients (10.5%). Of these, 15 were vaginal, 27 pelvic, 27 intra-abdominal and beyond the pelvis and 29 extra-abdominal. None of the studied variables was associated with an altered risk of vaginal or pelvic relapses in univariate analyses.

Poor differentiation, myometrial invasion (MI) ≥ 50% or higher, a tumor size of 3 cm or greater and abnormal peritoneal cytology were associated with an increased risk of intra-abdominal relapses. A tumor size of 3 cm or greater and abnormal peritoneal cytology predicted intra-abdominal relapses beyond the pelvis in multivariate analysis, whereas poor differentiation MI ≥ 50% and abnormal peritoneal cytology predicted extra-abdominal relapses. Compared to vaginal relapses, intra-abdominal relapses beyond the pelvis and extra-abdominal relapses were associated with worse disease-specific survival. Survival of patients with a pelvic relapse did not differ from that of patients with a vaginal relapse.

In the fourth study, the incidence of surgical site infection and its risk factors in EC were evaluated. In all, 912 women (78.4%) of the cohort 1164 EC patients who had had a hysterectomy had minimally invasive surgery. Ninety-four women (8.1%) were diagnosed with a surgical site infection. Twenty women (1.7%) had an incisional infection, and 74 (6.4%) had an organ/space infection. The associations of 17 clinicopathologic and surgical variables were tested by univariate analyses. Those variables that were identified as potential risk factors in univariate analyses (p < 0.15) were used in logistic regression models with incisional and organ/space infections as dependent variables. Obesity (body mass index ≥ 30 kg/m²), diabetes and long operative time (> 80th centile) were independently associated with a higher risk of incisional infection, whereas minimally invasive surgery was associated with a smaller risk. Smoking, conversion to laparotomy, and lymphadenectomy were associated with a higher risk of organ/space infection.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AH</td>
<td>Atypical hyperplasia</td>
</tr>
<tr>
<td>ASR</td>
<td>Age-standardized incidence rate</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BSO</td>
<td>Bilateral salpingo-oophorectomy</td>
</tr>
<tr>
<td>BT</td>
<td>Brachytherapy</td>
</tr>
<tr>
<td>CA-125</td>
<td>Cancer antigen, carbohydrate 125</td>
</tr>
<tr>
<td>CSI</td>
<td>Cervical stromal involvement</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CTCs</td>
<td>Circulating tumor cells</td>
</tr>
<tr>
<td>ctDNA</td>
<td>Circulating tumor DNA</td>
</tr>
<tr>
<td>CTNNB1</td>
<td>Catenin beta 1</td>
</tr>
<tr>
<td>DFS</td>
<td>Disease-free survival</td>
</tr>
<tr>
<td>DSS</td>
<td>Disease-specific survival</td>
</tr>
<tr>
<td>EC</td>
<td>Endometrial carcinoma</td>
</tr>
<tr>
<td>EEC</td>
<td>Endometrioid endometrial carcinoma</td>
</tr>
<tr>
<td>EH</td>
<td>Endometrial hyperplasia</td>
</tr>
<tr>
<td>EIN</td>
<td>Endometrioid intraepithelial neoplasia</td>
</tr>
<tr>
<td>ER</td>
<td>Estrogen receptor</td>
</tr>
<tr>
<td>ESGO</td>
<td>European Society of Gynaecological Oncology</td>
</tr>
<tr>
<td>ESMO</td>
<td>European Society for Medical Oncology</td>
</tr>
<tr>
<td>ESTRO</td>
<td>European Society for Radiotherapy &amp; Oncology</td>
</tr>
<tr>
<td>FIGO</td>
<td>International Federation of Gynecology and Obstetrics</td>
</tr>
<tr>
<td>LiCAM</td>
<td>L1 cell adhesion molecule</td>
</tr>
<tr>
<td>FinGOG</td>
<td>Suomen Gynecologisen Onkologian Erikoislääkärit</td>
</tr>
<tr>
<td>LND</td>
<td>Lymph node dissection</td>
</tr>
<tr>
<td>LNG-IUD</td>
<td>Intrauterine levonorgestrel-releasing device</td>
</tr>
<tr>
<td>LNMI</td>
<td>Lymph node metastasis</td>
</tr>
<tr>
<td>LVSI</td>
<td>Lymphovascular space involvement</td>
</tr>
<tr>
<td>MA</td>
<td>Megestrol acetate</td>
</tr>
<tr>
<td>MI</td>
<td>Myometrial invasion</td>
</tr>
<tr>
<td>MMR</td>
<td>Mismatch repair</td>
</tr>
<tr>
<td>MPA</td>
<td>Medroxyprogesterone acetate</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MSI</td>
<td>Microsatellite instability</td>
</tr>
<tr>
<td>NEEC</td>
<td>Non-endometrioid endometrial cancer</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative predictive value</td>
</tr>
<tr>
<td>OC</td>
<td>Oral contraceptive</td>
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<tr>
<td>OS</td>
<td>Overall survival</td>
</tr>
<tr>
<td>PCOS</td>
<td>Polycystic ovarian syndrome</td>
</tr>
<tr>
<td>PET/CT</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived growth</td>
</tr>
<tr>
<td>POLE</td>
<td>Exonuclease domain of DNA polymerase epsilon</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive predictive value</td>
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<tr>
<td>PR</td>
<td>Progesterone receptor</td>
</tr>
<tr>
<td>Pten</td>
<td>Phosphatase and tensin homolog</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SEC</td>
<td>Serous endometrial carcinoma</td>
</tr>
<tr>
<td>SSI</td>
<td>Surgical site infection</td>
</tr>
<tr>
<td>SLN</td>
<td>Sentinel lymph node</td>
</tr>
<tr>
<td>TCGA</td>
<td>The Cancer Genome Atlas Research Network</td>
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<tr>
<td>TGFB</td>
<td>Transforming growth factor-beta</td>
</tr>
<tr>
<td>TP53</td>
<td>Tumor protein 53 gene</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WPRT</td>
<td>Whole pelvic radiation therapy</td>
</tr>
<tr>
<td>2D-TVU</td>
<td>Two-dimensional transvaginal ultrasonography</td>
</tr>
<tr>
<td>3D-TVU</td>
<td>Three-dimensional transvaginal ultrasonography</td>
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</tbody>
</table>
Introduction

1 INTRODUCTION

Endometrial cancer (EC) is a malignant tumor arising from the inner lining of the uterine cavity. It is the most common gynecologic cancer in the developed countries (Torre et al., 2015). The cardinal symptom is uterine bleeding, which presents in the early phase of the disease (Goldstein et al., 2001).

Tumor dissemination is demonstrated in stages. Staging plays the utmost important role in planning the treatment and predicting the prognosis of the disease. The first extra-uterine spread is usually via the lymphatic vessels. The five-year survival rate is 80–90% in patients with local disease, but less than 60% in those with lymph node metastasis (LNM) (Lewin, Wright, 2011). The principal treatment for EC is hysterectomy and bilateral salpingo-oophorectomy (BSO). To decide the extent of surgery, patients with EC are classified as low-, intermediate- or high-risk according to predictive factors for LNM. The rationale of lymphadenectomy lies in prognostication and the legitimate triage of patients for adjuvant therapy. Currently, complete lymphadenectomy is advocated for staging and guidelines advise it for cases with high-risk features (Colombo et al., 2016). Selective lymph node dissection (LND), allowing the low-risk patients to sentinel lymph node mapping, or forgoing lymphadenectomy saves the low-risk patients from systematic LND-related complications such as nerve and vessel damage (Geppert et al., 2018).

According to two prospective randomized trials, lymphadenectomy does not improve the survival of patients with low-risk EC (ASTEC study group et al., 2009, Benedetti Panici et al., 2008). Kim et al. demonstrated that systematic lymphadenectomy, defined as the removal of ≥10–11 lymph nodes, was associated with limited survival benefit in patients with low-risk endometrial cancer, but it resulted in improved overall survival (OS) in patients with intermediate- or high-risk EC (H. S. Kim et al., 2012).

To optimize risk assessment, various prognostic factors have been combined to form prognostic models that demonstrate the probability of LNM. An ideal risk-stratification method is both accurate and associated with a decent rate of lymphadenectomy, and may even predict patient survival.

This study was conducted to evaluate the reliability of currently available risk-stratification methods and to develop new, clinically applicable methods to allow decisions about primary surgery and possible adjuvant therapy and to gauge the EC patient’s risk for surgery-related mortality and disease-related long-term morbidity.
2 REVIEW OF THE LITERATURE

2.1 EPIDEMIOLOGY

EC is the most prevalent gynecological cancer in developed countries with 170 000 new cases annually. Globally there are 320 000 new cases per year (Torre et al., 2015). North America possesses the highest incidence (19.1 per 100,000) and South-Central Asia the lowest (2.7 per 100,000), leaving Northern Europe with an intermediate-high incidence (14.1 per 100,000) (Ferlay et al., 2015).

In Finland, there are approximately 850 new EC cases and 185 deaths annually. The age-standardized incidence rate (ASR) is 13.4 per 100 000, and mortality is 2 per 100 000. The average age at diagnosis is 69 years, and only 5% of ECs are diagnosed in women younger than 50 years of age (Finnish Cancer Registry, 2016).

International EC trends were extracted from the population-based cancer registries in 43 populations from 1978–2013 (Lortet-Tieulent et al., 2018). The EC incidence has increased in all ages in more than half of the populations, most rapidly in countries with the lowest rates. A significant decreased trend in ASR was observed in Slovenia, Austria and Sweden. Trends in Norway, Finland and France express a downward tendency among premenopausal women, but rates are stable in elderly women.

Various combined risk factors and preventive aspects influence the incidence rates in different countries. Spreading obesity and longevity are responsible for most of the increase (Arnold et al., 2015, Onstad, Schmandt & Lu, 2016, Pearson-Stuttard et al., 2018) . Altering reproductive patterns toward fewer childbirths and nulliparity may contribute to the increased EC rate, particularly in low-fertility countries (Lortet-Tieulent et al., 2018)

The prognosis of endometrial cancer is good when the diagnosis is made at an early stage. The five-year relative survival rate in localized cancer reaches 95%, regional cancer 68% and metastatic cancer 17% (Siegel, Miller & Jemal, 2017).
Figure 1. New endometrial cancer cases (incidence) and mortality among all Finnish women, 1953–2015. Data from the Finnish Cancer Registry, with permission.
2.2 ETIOLOGY

2.2.1 HISTOLOGICAL CLASSIFICATION

The World Health Organization’s (WHO) histological classification of EC (Kurman et al., 2014) is summarized in Table 1.

Table 1. Histologic classification and incidence of EC (modified from the WHO classification) (Kurman et al., 2014)

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrioid</td>
<td>70–80%</td>
</tr>
<tr>
<td>Mucinous</td>
<td>1–9%</td>
</tr>
<tr>
<td>Serous</td>
<td>5–10%</td>
</tr>
<tr>
<td>Clear cell</td>
<td>2%</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>Mixed cell</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Dedifferentiated</td>
<td>Uncommon</td>
</tr>
</tbody>
</table>

The endometrioid-type EC is subdivided into three grades: well (grade 1), moderately (grade 2) and poorly (grade 3) differentiated carcinomas conforming to the extent of solid, non-gland forming clusters of neoplastic cells. Grades 1 and 2 together constitute up to 85% of endometrioid endometrial cancer (EEC) (Zaino et al., 1995).
2.2.2 DICHOTOMOUS CLASSIFICATION

The division of EC into type I and type II subgroups is based on clinicopathological findings (Bokhman, 1983). Type I covers about 80% of all ECs and represents estrogen-related well-differentiated endometrioid histology. It is commonly diagnosed at an early stage in obese younger women. Type II EC is predominantly poorly differentiated endometrioid carcinoma or non-endometrioid carcinoma that more frequently occurs in the elderly. It is estrogen-independent and associated with a higher risk of metastasis and poorer prognosis (Bokhman, 1983). Type II ECs constitute around 15–20% of all ECs but cause 40% of deaths (Moore, Fader, 2011, Morice et al., 2016). This classification is useful as it underlines differences in the pathogenesis of various types of endometrial carcinoma, but it is not applicable to clinical decision-making.

The dualistic model has triggered diverse studies demonstrating that EC appears to be a heterogeneous and convoluted disease from the epidemiologic, pathological, clinical and molecular perspectives (Suarez, Felix & Cohn, 2017).

2.2.3 GENOMIC CHARACTERIZATION

The Cancer Genome Atlas Research Network (TCGA) elucidated exomes of 307 endometrioid-, 53 serous- and 13 mixed histology–type EC patients. This landmark study classified EC into four prognostically meaningful distinct genomic categories based on the integration of mutational spectra, microsatellite instability status and copy-number alterations: 1) POLE ultramutated, 2) MSI hypermutated 3) copy-number low, frequently CTNNB1 mutated endometrioid tumors and 4) copy-number high, characterized by TP53 mutation (Cancer Genome Atlas Research Network et al., 2013). In a study of high-grade EEC patients, the disease-specific survival at 48 months indicated an excellent 100% for the POLE-mutated group, 82% for the MSI group, 78% for the copy-number low group and only 43% for the copy-number high group (Piulats et al., 2017).

The transPORTEC research group scrutinized the clinical outcomes by all the molecular subgroups perceiving a five-year recurrence-free survival of 93% for the POLE-mutant group and 95% for the MSI group in contrast to 52% for copy-number low and 42% for the P53-mutant group (Stelloo et al., 2015). ProMisE, Proactive Molecular Risk Classifier for Endometrial Cancer, is a recently developed and validated utilitarian molecular classification implement. Like TCGA, it distinguishes four prognostically specific molecular subtypes, yet it is additionally applicable to preoperative diagnostic on specimens (Komnoss et al., 2018). Compared to the p53 wild-type subgroup (copy-number low TCGA
classification), microsatellite instable, *POLE*-mutant and *P53*-mutant groups had overall survival hazard ratios of 2.21, 0.78 and 3.54, respectively. Progression-free survival (PFS) hazard ratios were 3.30, 0.51 and 7.84 (Talhouk et al., 2017). The genomic characterization supplies great prognostic and predictive potential for risk stratification of EC together with clinicopathological parameters.

2.2.4 RISK AND PREVENTIVE FACTORS

Exposure to endogenous or exogenous estrogen is the major generator of EC. Obesity, early menarche, late menopause, nulliparity, polycystic ovarian syndrome (PCOS) and estrogen-secreting tumors are risk factors for EC via an overload of estrogen (Arnold et al., 2015, Setiawan et al., 2013). Entrenched epidemiological studies show that a 5 kg/m² increase in body mass index (BMI) is associated with a three-fold higher risk of EC (Renehan et al., 2008, Zhang et al., 2014). High BMI answers for over one-third (34%) of the worldwide EC incidence and practically half (48%) of the cases in North America (Arnold et al., 2015).

EC risk is associated with metabolic syndrome and its component factors, not only obesity but also impaired fasting glucose, high blood pressure, high triglycerides (Trabert et al., 2015) and diabetes (Pearson-Stuttard et al., 2018, Nead et al., 2015).

In the 1970s, exogenous administration of estrogens only was found to greatly increase the risk of EC (McDonald et al., 1977, Smith et al., 1975).

The selective estrogen receptor modulator tamoxifen operates in the postmenopausal endometrium through estrogenic and genotoxic pathways, enhancing the risk of less favorable morphological subtypes of EC (Hu, Hilakivi-Clarke & Clarke, 2015, Pinkerton, Thomas, 2014). The relative risk (RR) of EC with tamoxifen users for 2–5 years is 2.0, whereas for users for five years or more, RR is 6.6 compared with non-users(Bergman et al., 2000). Long-term tamoxifen users have a poorer prognosis of EC due to adverse histology and advanced stages (Bergman et al., 2000).

Combined estrogen-progesterone oral contraceptives (OCs) (Michels et al., 2018) and smoking (Jacob et al., 2018), as well as high parity and late age at first and last childbirth (Bevier, Sundquist & Hemminki, 2011), correlate with a declined risk of EC. According to a meta-analysis, OC use in developed countries may have prevented around 400 000 EC cases during the past 50 years (Collaborative Group on Epidemiological Studies on Endometrial Cancer, 2015).
2.2.5 HEREDITARY PREDISPOSITION

Almost six percent of ECs are based on inherited cancer predisposition syndromes, and Lynch syndrome (LS) covers most of them (Lu, 2008). LS is inherited in an autosomal-dominant manner by germline mutations in one of the four mismatch repair (MMR) genes (MLH1, MSH2, MSH6 and PMS2) or the epithelial cell adhesion molecule (EPCAM), which is the regulator of MSH2 (Backes, Cohn, 2011). Individuals with Lynch syndrome are at amplified lifetime risk for several cancers, including colorectal, endometrial and ovarian cancers. The cumulative lifetime risk of EC is ∼60% (27–71%) depending on the MMR gene mutated (Bartosch, Clarke & Bosse, 2018). The proportion of type II cancers is higher in LS compared to non-LS endometrial carcinomas (Broaddus et al., 2006). Hysterectomy and bilateral salpingo-oophorectomy (BSO) are suggested as risk-reducing interventions for LS carriers after childbearing (Ring et al., 2017, Committee on Practice Bulletins-Gynecology, Society of Gynecologic Oncology, 2014).

Germline mutations in POLD1 and POLE genes result in colonic polyps and subsequent high colorectal cancer risk, with a likewise increased EC risk (Briggs, Tomlinson, 2013). Cancers caused by these germline mutations may express features similar to Lynch syndrome cancers such as microsatellite instability. POLD1 and POLE mutations are inherited in an autosomal dominant pattern (Ring et al., 2017).

Uncommon germline variations in the phosphatase and tensin homolog (PTEN) gene, Cowden syndrome, carry up to 28% lifetime risk of EC, as well as increased risk of breast cancers, thyroid cancer and renal cell cancers (Tan et al., 2012). Cowden syndrome is part of a larger genetic syndrome known as PTEN hamartomatous tumor syndrome that is inherited in an autosomal dominant pattern and can display reduced penetrance and variable expressivity even within a family (Ring et al., 2017).

BRCA mutations have been associated with EC risk, specifically of more aggressive types, such as serous-type endometrial cancer (SEC) (Bruchim et al., 2010, Shu et al., 2016). However, EC was not more prevalent than expected in a larger BRCA-study population, although the specific occurrence of high-risk EC was not statistically analyzed (Mersch et al., 2015).
2.3 PATHOGENESIS

2.3.1 PRECURSOR LESIONS

Endometrial hyperplasia is a physiological, polyclonally proliferative reaction to excessive estrogen. In addition to this preliminary estrogenic stimulation, a localized monoclonal outgrowth of genetically altered endometrial glands is required to construct AH, which progressively mutates into carcinoma (Mutter, 2000). The WHO histological classification is shown in Table 2 (Kurman et al., 2014).

**Table 2.** Endometrial Diagnostic Terminology after the WHO 2014 classification

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>Cancer Risk</th>
<th>Topography</th>
<th>Functional Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial Hyperplasia without Atypia</td>
<td>1–5x</td>
<td>Diffuse</td>
<td>Prolonged estrogen effect</td>
</tr>
<tr>
<td>AH; Atypical Hyperplasia /EIN; Endometrioid Intraepithelial Neoplasia</td>
<td>45x</td>
<td>Focal progressing to diffuse</td>
<td>Precancerous monoclonal neoplasm</td>
</tr>
<tr>
<td>Endometrial adenocarcinoma, endometrioid type, well differentiated</td>
<td></td>
<td>Focal progressing to diffuse</td>
<td>Monoclonal malignant neoplasm</td>
</tr>
</tbody>
</table>

Modified from the WHO (Kurman et al., 2014), and Mutter et al. (ESGO, European Society of Gynaecological Oncology, 2009)
Uterine serous cancer is the most frequent form of estrogen non-related EC. It is distinguished by its uniformly manifest cytologic atypia and is high-grade malignant by definition. The grounding non-neoplastic endometrium is extensively atrophic (Wheeler et al., 2000). Serous endometrial intraepithelial carcinoma (SEIC) is thought to represent the precursor lesion of serous carcinoma. Microscopically, it is composed of adenopapillary structures lined by the epithelium with prominent nuclear atypia but without evidence of myometrial stromal invasion (Ambros et al., 1995, Lax, 2017).

2.4 DIAGNOSIS

2.4.1 DIAGNOSTIC METHODS

Abnormal uterine bleeding is the cardinal symptom (90%) of EC frequently presenting in an early phase of the disease (Goldstein et al., 2001). Abdominal soreness and enlargement occur in more advanced stages (Moric et al., 2016). Depending on individual risk factors, EC is the cause of 1–14% of postmenopausal bleeding (Clarke et al., 2018, Gupta et al., 2002, Smith-Bindman et al., 1998, Tabor, Watt & Wald, 2002).

Endometrial sampling

EC diagnosis rests on the histopathological evaluation of an endometrial sample material. Preoperative sampling may be performed by office endometrial biopsy, hysteroscopic biopsy or uterine dilatation and curettage. Attempted office endometrial biopsy succeeds around 90% of the time (Dijkhuizen et al., 2000).

Restricted attainable tissue challenges the diagnostics. In a retrospective study of 139 women with postmenopausal bleeding, a minimum cut-off value of 35 mm² required classifying an endometrial office biopsy sample as conclusive for the right diagnosis (Reijnen et al., 2017). The main problem is the simultaneous presence of both AH and carcinoma and insufficient sampling of the latter one. Therefore, not only the amount but also the preciseness of sampling is imminent. Visser et al. published a review and meta-analysis on the accuracy of preoperative endometrial sampling and final diagnosis in EC; the pooled percentage of overall agreement was 0.67 (95% CI 0.60–0.75), including 5055 patients. Hysteroscopic biopsies displayed the highest agreement, followed by office endometrial biopsy and dilatation and curettage. The lowest agreement rate was found for grade 2
carcinomas (0.61, 95% CI 0.53–0.69). Downgrading was found in 25% of cases, of which 26% were clinically relevant, and upgrading was found in 21%. Altogether, 8% of upgrading was clinically relevant (Visser et al., 2017).

**Imaging**

Imaging modalities are used to estimate the extent of the disease to optimize tailored operative treatment. Imaging is further run for planning adjuvant treatment and identifying the postoperative residual disease in high-risk patients, monitoring and noticing recurrent disease, and making post-treatment observations of asymptomatic high-risk patients (Lin et al., 2018).

**Ultrasound**

Transvaginal ultrasonography is a suitable first-line examination method of postmenopausal bleeding given that an endometrial thickness of 4 mm or less carries more than 99% negative predictive value for EC (ACOG Committee Opinion, 2018, Karlsson et al., 1995, Wong et al., 2016). Nevertheless, histological evaluation of the endometrium should be elicited for women with persistent or recurrent bleeding or individual risk factors for EC regardless of endometrial thickness (ACOG Committee Opinion, 2018, Practice Bulletin, 2015).

Transvaginal ultrasound is advocated within the preoperative clinical examination of the EC patient, identifying the size of the tumor, possible myometrial invasion (MI) and cervical stromal invasion (CSI); these parameters influence the likelihood of high-risk disease and advanced stage (Colombo et al., 2016). Two-dimensional transvaginal ultrasonography (2D-TVU) has a moderate diagnostic performance of MI with an overall pooled sensitivity of 82% and a specificity of 81% according to a meta-analysis (Alcazar et al., 2015). So far, the three-dimensional transvaginal ultrasonography (3D-TVU) shows lower agreement, reliability, and accuracy in MI and CSI evaluation than 2D-TVS (Christensen et al., 2016, Green et al., 2018).
Magnetic resonance imaging

Magnetic resonance imaging (MRI) is adopted to appraise the extent of local disease and any other anatomical feature that may impact the extent of primary surgery. Meta-analysis on the diagnostic accuracy of MRI for MI and CSI demonstrate a resembling sensitivity of 81%, specificity 89%, PPV 78% and NPV 90% (Luomaranta, Leminen & Loukovaara, 2015), with meta-analyses on ultrasonography (Alcazar et al., 2015). Also, MRI and ultrasound methods analyzing the same patients equally express MI and CSI (Antonsen et al., 2013, Epstein, Blomqvist, 2014, Savelli et al., 2008). Only slightly higher accuracy of MRI compared to 2D-TVS and 3D-TVS was found in a study by Christensen et al. (Christensen et al., 2016). MRI may also distinguish metastatic lymph nodes based on their morphology and size, but the sensitivity is low, around 45% (Luomaranta, Leminen & Loukovaara, 2015).

Computed tomography

Computed tomography (CT) possesses high multiplanar spatial resolution that is advantageous in visualizing the whole body for expanded lymph nodes and gross soft tissue masses. Established 10 mm threshold criteria for suspicious metastatic nodes cause limitations, considering that the overall reported sensitivity in detecting nodal metastasis is scarce at 40%. Diversely, in a study on 32 lymph node-positive patients, 54% of the positive lymph nodes measured less than 10 mm, and 29% of negative lymph nodes measured more than 10 mm (Lin et al., 2018, Reich et al., 1996). In patients with multiple positive nodes, 85% of patients had at least one positive node larger than 10 mm (Ayhan et al., 1995, Lin et al., 2018).

Positron emission tomography

Positron emission tomography (PET/CT) detects glucose consumption in cells (Faria et al., 2018). This metabolic activity is higher in malignant tumors than in normal cells. The most commonly used radiotracer that gets trapped within tumor cells after injection is 18F-fluorodeoxyglucose (FDG). CT scanning simultaneously images the tumor sites (Faria et al., 2018). PET-CT, CT, and MRI have similar accuracies in discovering lymph node metastases in EC (Antonsen et al., 2013, Kitajima et al., 2009, Park et al., 2008). PET-CT, being the absolute most expensive and effortful imaging modality, is usually not applied in the preoperative setting. PET–CT may be beneficial in determining whether the
extent of recurrence is localized or disseminated (Epstein, Blomqvist, 2014, Lin et al., 2018)

Gross visualization

Intraoperative gross visual examination of a dissected hysterectomy specimen provides a possibility to measure the macroscopic tumor size and MI. Gross visual assessment of myometrial invasion has an MI accuracy of up to 85% (Mao et al., 2008, Marcickiewicz, Sundfeldt, 2011), which is comparable to TVUS, MRI and PET/CT.

2.4.2 STAGING

EC has four possible dissemination pathways that may present alone or in combination: contiguous, peritoneal (via exfoliation), lymphatic and hematogenous (Lax, Tamussino & Lang, 2016). The primary local extension affects the myometrium, and the lymph system is the main channel to the further extrauterine stage. The lymphatic system of the corpus uteri is constructed of three main lymphatic trunks: utero-ovarian (infundibulopelvic), parametrial, and presacral. They collectively drain into the hypogastric/internal iliac, external iliac, common iliac, presacral and para-aortic nodes (Amant et al., 2018). Direct metastases to the para-aortic lymph nodes are infrequent, even though a direct lymphatic route from the uterus through the infundibulopelvic ligament has been proposed in anatomical and sentinel lymph node research (Amant et al., 2018, Geppert et al., 2017).

Tumor dissemination is demonstrated in stages. Staging plays the utmost important role in planning the treatment and predicting the prognosis of the disease. As of 1988, EC is surgically staged. The International Federation of Gynaecology and Obstetrics (FIGO) published a current official update on staging in 2009, shown in Table 3.

In 2017, the American Joint Committee on Cancer (AJCC) published its most recent (its eighth) update on the tumor node metastasis system (TNM) to address individual tumor cells (ITCs) in lymph nodes for gynecologic cancers. According to the staging manual, the existence of ITCs should be marked pNo(i+), but it does not affect the lymph node status (Olawaiye, Mutch, 2018). Lymph node
metastases are listed by their size in three groups; ITC < 0.2 mm, micrometastasis 0.2–2 mm and macrometastasis > 2 mm. ITCs and macrometastasis are jointly assigned as low-volume metastasis (LVM) (Holloway et al., 2016, St Clair et al., 2016, Touboul et al., 2013). As a result of ultrastaging during sentinel lymph node mapping, the detection rate of LVM has increased from 0.8 to 8.0% depending on tumor grade and MI (C. H. Kim et al., 2013), yet the understanding of the clinical significance of LVM remains incomplete thus far (Olawaiye, Mutch, 2018, Todo et al., 2016).
<table>
<thead>
<tr>
<th>FIGO</th>
<th>TNM</th>
<th>Definition</th>
<th>5-year survival %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td>Tumor confined to the uterine corpus</td>
<td></td>
</tr>
<tr>
<td>IA</td>
<td>T1aN0Mo</td>
<td>No or less than half of myometrium invasion</td>
<td>96</td>
</tr>
<tr>
<td>IB</td>
<td>T1bN0Mo</td>
<td>Invasion equal to or more than half of the myometrium</td>
<td>87</td>
</tr>
<tr>
<td>II</td>
<td>T2N0Mo</td>
<td>Tumor invades cervical stroma but does not extend beyond the uterus</td>
<td>80</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>Local and/or regional spread</td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>T3aN0Mo</td>
<td>Tumor invades serosa of the uterus and/or adnexae</td>
<td>48</td>
</tr>
<tr>
<td>IIIB</td>
<td>T3bN0Mo</td>
<td>Vaginal and/or parametrial involvement</td>
<td>53</td>
</tr>
<tr>
<td>IIIC1</td>
<td>T1-3N1Mo</td>
<td>Metastases to pelvic lymph nodes</td>
<td>60</td>
</tr>
<tr>
<td>IIIC2</td>
<td>T1-3N1Mo</td>
<td>Metastases to para-aortic lymph nodes with or without positive pelvic nodes</td>
<td>53</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td>Tumor invades bladder and/or bowel mucosa and/or distant metastases</td>
<td></td>
</tr>
<tr>
<td>IVA</td>
<td>T4AnyNMo</td>
<td>Tumor invasion of bladder and/or bowel mucosa</td>
<td>57</td>
</tr>
<tr>
<td>IVB</td>
<td>AnyT</td>
<td>Distant metastases incl. intra-abdominal metastases and/oringuinal nodes</td>
<td>16</td>
</tr>
</tbody>
</table>

Modified from FIGO, the American Joint Committee on Cancer (AJCC) and the WHO (Pecorelli, 2009, Edge, Edge, 2010, Kurman et al., 2014, Werner et al., 2012).
2.5 RISK STRATIFICATION

2.5.1 RATIONALE

To decide the extent of surgery, patients with EC are classified as low-, intermediate- or high-risk according to predictive factors for LNM (Colombo et al., 2016). The rationale of lymphadenectomy lies in prognostication and the legitimate triage of patients for adjuvant therapy. Currently, complete lymphadenectomy is advocated for staging and guidelines advise it for cases with high-risk features (Colombo et al., 2016), shown in Table 4. Selective LND, allowing the low-risk patients to undergo sentinel lymph node mapping or to forgo lymphadenectomy, saves the low-risk patients from the complications related to systematic LND, such as nerve and vessel damage (Geppert et al., 2018).

2.5.2 LYMPHADENECTOMY

The definition of an adequate lymphadenectomy has not been standardized. Presently, it includes pelvic and para-aortic nodal execution. Systematic pelvic lymphadenectomy often means node removal from the level of the external iliac vessels and the caudal half of the obturator fat pad anterior to the obturator nerve. Para-aortic lymphadenectomy up to the renal vessels has been suggested since the Mayo Clinic research on 281 patients with endometrial cancer who underwent lymphadenectomy. Of these, 22% of patients with high-risk disease had lymph node metastases: 51% had both positive pelvic and para-aortic nodes, 33% had positive pelvic lymph nodes only and 16% had isolated para-aortic lymphadenopathy with 77% of patients having positive nodes above the inferior mesenteric artery (IMA) (Mariani et al., 2008). A survey by the Society of Gynecologic Oncology (SGO) noted that only 11% of responding clinicians proceeded with para-aortic evacuation to the renal vessels (Soliman et al., 2010). Para-aortic nodes may be positive in the absence of positive pelvic nodes; the rate of these skip-metastases is around 1–3%, with the exception of patients with deeply invasive high-grade EC, in whom this percentage is higher than 10% (Abu-Rustum et al., 2009, Kumar et al., 2014).

The therapeutic benefits of lymphadenectomy are controversial. Analysing data from nine trials, Kim et al. demonstrated that systematic lymphadenectomy, defined as the removal of $\geq 10-11$ lymph nodes, was associated with limited survival benefit in patients with low-risk endometrial cancer, but resulted in improved overall survival (OS) in patients with intermediate- or high-risk EC (H. S. Kim et al., 2012). Two prospective randomized trials studying the effect of
pelvic lymphadenectomy on low-risk patients showed improved staging but not improved survival of LND (ASTEC study group et al., 2009, Benedetti Panici et al., 2008).

2.5.3 Sentinel Lymph Node Mapping

SLN mapping presents midway between eradication and omission of node dissection. SLN mapping involves an injection of a colored dye or radioisotope tracer at two to four sites of the cervix at the start of a laparoscopic EC operation. Currently, a fluorescent dye called indocyanine green (ICG) is the preferred method (Abu-Rustum, 2014, Rossi, Ivanova & Boggess, 2012). With a near-infrared camera, the lymphatic veins and first lymph nodes (the sentinels) are visualized on both sides and consequently operated on following minimal invasive protocol (Staley, Sullivan & Rossi, 2017). Barlin et al. published prospective data in 2012 with an algorithm for SLN mapping, underlining the importance of the removal of any suspicious node along with site-specific lymphadenectomy for failed mapping (Barlin et al., 2012).

In EEC with superficial MI, the use of an SLN algorithm compared to selective nodal assessment based on tumor characteristics has shown a higher detection rate of stage IIIC1 disease and a similar detection rate of stage IIIC2 disease, with no evident concession in oncologic outcomes (Zahl Eriksson et al., 2016). Likewise, the detection of stage IIIC1 disease in patients with any grade EEC and deep MI show similar rates with SLN assessment and full LND (Ducie et al., 2017, Schlappe et al., 2018) without comprising the oncologic outcome (Schlappe et al., 2018). The SLN concept in low-risk endometrial cancer patients identifies the 5–8% with LNM, partially LVM (Chi et al., 2008, Hagen et al., 2016, C. H. Kim et al., 2013, Persson et al., 2017). Additionally, successful SLN mapping avoids the consequences of preoperative under-staging (Leitao et al., 2008).

2.5.4 Prognostic Factors for Disease Spread and Outcome

Uterine factors

Pathologic features of the primary tumor were first studied in the prediction of LNM over 30 years ago. Poor differentiation, deep MI and cervical stromal invasion (CSI) were found to anticipate lymphatic dissemination (Boronow et al., 1984, Creasman et al., 1987). With one uterine risk feature present, the incidence
of pelvic LNM was up to 27% and para-aortic LNM up to 19%, rising further with multiple risk factors (Creasman et al., 1987).

Tumor size has been identified as a prognostic factor for LNM (Canlorbe et al., 2016, Schink et al., 1991). The size is measured in final pathology analysis, but along with the MI, it can also be estimated intraoperatively by frozen section analysis. Frozen section technique requires appropriate pathology expertise and may therefore be variable (Kumar et al., 2011). Furthermore, tumor size can be reliably assessed intraoperatively by directly measuring the maximal diameter of the largest identified lesion by gross visualization (AlHilli et al., 2013).

Lymphovascular space invasion (LVSI) is defined as tumor emboli within blood vessels and/or lymphatic channels. It has been identified as an independent predictor of recurrences and poor outcomes owing to its high association with nodal metastases (Aristizabal et al., 2014, Guntupalli et al., 2012) but also in the absence of nodal disease (Cusano et al., 2018, Jorge et al., 2016).

The survival of node-negative patients with multiple uterine risk factors has been found to be poorer than (Kwon et al., 2009) or almost as poor as (Barrena Medel et al., 2011) that of node-positive patients without any risk factors. By the same token, uterine risk factors have a negative impact on survival, even in the presence of nodal metastases (Barrena Medel et al., 2011, Kwon et al., 2009, Milgrom et al., 2014, Vargas et al., 2014).

Peritoneal cytology was included as a staging procedure in bygone guidelines, but it is no longer obligatory (Pecorelli, 2009). However, several retrospective studies (Garg et al., 2013, Han et al., 2014) indicate abnormal peritoneal cytology to have a prognostic value, especially in non-endometrioid endometrial cancer (NEEC). Furthermore, a recent retrospective study on 1668 stage I–II EEC patients showed that abnormal peritoneal cytology was independently associated with decreased survival and distant recurrence (Matsuo et al., 2018). Since abnormal cytology may still add to the effect of other risk factors, FIGO, AJCC and the joint consensus of the European Society for Medical Oncology (ESMO), European Society for Radiotherapy & Oncology (ESTRO) and European Society of Gynaecological Oncology (ESGO) recommend continuing to obtain peritoneal cytology during surgery (Colombo et al., 2016, Koh et al., 2018).

The prognosis of EC is profoundly dependent on stage, histological grade and age (Colombo et al., 2016). The revised 2010 FIGO surgical classification system advocates for complete surgical staging, although a recent Cochrane review concluded that this pervasive surgery did not contribute to improved outcomes in low-risk EC (Frost et al., 2017). The latest ESMO-ESGO-ESTRO recommendations to the extent of surgical treatment are stated in Table 4.
**Table 4.** Preoperative profiling for endometrial cancer

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Criteria</th>
<th>Primary treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low</strong></td>
<td>MI &lt; 50%, Endometrioid G1-2</td>
<td>H+BSO</td>
</tr>
<tr>
<td><strong>Intermediate</strong></td>
<td>MI &lt; 50%, Endometrioid G3 MI ≥ 50% G1-2</td>
<td>H+BSO+LND</td>
</tr>
<tr>
<td><strong>High</strong></td>
<td>MI ≥ 50%, Endometrioid G3, CSI, Nonendometrioid</td>
<td>H+BSO+LND + Omentectomy in SER</td>
</tr>
</tbody>
</table>

Adopted from ESMO-ESGO-ESTRO guidelines (Colombo et al., 2016) and FIN-GOG (FIN-GOG, 2018) guidelines. MI=myometrial invasion, H=hysterectomy, BSO=bilateral salpingo-oophorectomy, LND=lymphadenectomy (pelvic and para-aortic), CSI=cervical stromal invasion, SER=serous endometrial cancer.

**Demographic factors**

Obesity has been related to better disease-specific survival in EC (Kwon et al., 2009) but discordantly, also not to influence survival (Akbayir et al., 2012, Crosbie et al., 2012).

A study by Ko et al. found that diabetes correlated with worse recurrence-free survival and overall survival in Type I ECs but was not associated with type II outcomes (Ko et al., 2014).

An age higher than 65 years in early-stage disease has been found to be an independent prognostic factor for poorer disease-specific survival than the expectation for younger women (Benedetti Panici et al., 2014), even though age is not associated with the risk of nodal dissemination (Kwon et al., 2009).
**Biochemical factors**

Preoperative hematological parameters for anemia, thrombocytosis, and leucocytosis predict advanced disease and meager disease-specific survival (Njolstad et al., 2013). A registry-based primary care cohort study showed up to three years’ prediagnostic thrombocytosis to be independently associated with advanced EC and increased all-cause and cancer-specific mortality (Andersen et al., 2015). The interplays between platelets and cancer cells activate TGFβ-signaling, subsequently enhancing cancer metastasis (Cho et al., 2012, Labelle, Begum & Hynes, 2011). Furthermore, activated platelets engender the release of tumor growth factors and chemokines like platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), which stimulate cancer growth and metastasis (Li, 2016, Tesfamariam, 2016). Moreover, thrombocytes protect cancer cells from the immune clearance by natural killer cells, which also accelerates metastasis (Tesfamariam, 2016).

Cancer antigen 125 (CA-125) is an epithelial-cell surface antigen expressed in different types of tumor cells. EC-elevated preoperative concentrations of CA-125 predict the risk for extra-uterine disease and poor prognosis (Chen et al., 2011, Nicklin et al., 2012). Routine control of CA-125 during follow-up after treatment does not affect patient outcome, and it is not advised in SGO recommendations (Salani et al., 2017, Schwartz et al., 2017).

**Risk models**

To optimize risk assessment, various prognostic factors have been combined to form prognostic templates that demonstrate the probability of LNM. Nomograms aid in planning surgery and adjuvant therapy depending on the parameters utilized. So far, only a few practicable nomograms that facilitate the decision on lymphadenectomy within the primary surgery have been developed.

Among the most universally used risk models includes one developed by Mariani et al. in 2000; the Mayo low-risk criteria, named after the Mayo Clinic, include tumors with grade 1–2 endometrioid histology, myometrial invasion ≤ 50%, and diameter ≤ 2 cm according to frozen section analysis (Mariani et al., 2000). The proportion identified as low-risk patients is 27% (Mariani et al., 2008). Only 1.8% of the patients with Mayo criteria low-risk tumor were identified with LNM in a retrospective multicenter study (Convery et al., 2011). Subsequently, the Mayo Clinic’s Bogani et al. developed a 5-category risk-stratification system where noninvasive EEC regardless of size and grade also count as low-risk cases (Bogani et al., 2014).
Cox Bauer et al. introduced a method that includes the depth of MI and tumor size as parameters for identifying patients at low risk for LNM. The findings are based on the final pathology, but the variables were proposed as reliable for intraoperative estimation. Low-risk criteria with MI $\leq 33\%$ and diameter $\leq 50$ mm, regardless of grade met 43% of patients, allowing them to forgo LND with a 0% false negative rate (Cox Bauer et al., 2016).

**Biomarkers, genetic alterations**

Malignant development in human cancer is invariably induced by genetic and epigenetic alterations that disarray cell growth and death. Diverse molecular abnormalities result in different pathogenic pathways.

*PTEN*

Inactivation of the phosphatase and tensin homolog (*PTEN*), the tumor suppressor gene, is the most frequent genetic deformity in EEC, existing in over 80% of EEC specimens and 55% of precancerous lesions (Mutter et al., 2000, Philip et al., 2017). *PTEN* is a negative regulator of the PI3K/AKT/mTOR pathway (Cheung et al., 2011). Loss of *PTEN*, mostly by mutation, appears early in the EEC neoplastic process as a reaction to a surplus of estrogens not opposed by progestins in the endometrium (Mutter et al., 2001, Baak et al., 2005).

*PTEN* mutation is rare, just 10% in NEEC (Samarnthai, Hall & Yeh, 2010), and thus, it serves as a beneficial diagnostic marker in differentiating EEC from NEEC (Hussein et al., 2016). Loss of *PTEN* is related to improved survival in EC (Mackay et al., 2010). Inhibition of the PI3K/AKT/mTOR pathway shows a sensitization of the tumor cell line to PARP inhibitors (Philip et al., 2017) and mTOR inhibitors (Aghajanian et al., 2018, Arend et al., 2018).

*POLE*

Polymerase proofreading identifies and corrects mispaired bases along DNA replication. Missense mutations in these exonuclease domains of the major replicative DNA polymerase genes *POLE* and *POLD1* result in genomic instability that expresses as tumors with an extraordinary number of mutations, known as ultramutation (Rayner et al., 2016). Somatic POLE mutations are found in 7–12%
Review of the literature

of ECs (Cancer Genome Atlas Research Network et al., 2013, Church et al., 2013), where they evolve early and may exist already in AH/EIN (Temko et al., 2018).

Somatic POLE mutations in EC correlate with augmented tumor immunogenicity, a fine response to immune checkpoint inhibitors (Eggink et al., 2016, Howitt et al., 2015, van Gool et al., 2015) and an excellent prognosis (Cancer Genome Atlas Research Network et al., 2013, Talhouk, McAlpine, 2016).

Microsatellite instability

Inactivation of the mismatch repair (MMR) mechanism leads to microsatellite instability (MSI), the presence of small insertions and deletions at repetitive DNA. MSI is an early event, mostly in type I endometrial tumorigenesis. MSI appears in around 15–45% (MacDonald et al., 2000) of sporadic ECs and is the leading cause of hereditary EC (Bartosch, Clarke & Bosse, 2018, Meyer, Broaddus & Lu, 2009). Germline mutations occur in the four MMR genes MLH1, MSH2, MSH6, and PMS2, while the epigenetic impairment predominantly arises from the silencing of MLH1 by promoter hypermethylation (Salvesen et al., 2000, Simpkins et al., 1999).

MSI tumors present an enhanced immune cell infiltration with suggested notable sensitivity to checkpoint inhibitors (Cancer Genome Atlas Research Network et al., 2013, Eggink et al., 2016, Yamashita et al., 2017). The prognostic and predictive significance of MSI status and value of identifying patients at higher risk of LS has prompted guidelines for routine testing by MMR-IHC or MSI assay in all EC patients (Committee on Practice Bulletins-Gynecology, Society of Gynecologic Oncology, 2014).

DNA ploidy

Assessment of DNA ploidy status has been associated with prognosis in various epithelial cancers (Merkel, McGuire, 1990). In EC, aneuploidy has been reported to mark aggressive disease and meager survival (Pradhan et al., 2012).

Preoperative nondiploid curettage material showed a marginally elevated risk of LNM in a Norwegian study of 568 patients (Njolstad et al., 2015). A more recent population-based register study demonstrated no significant association between DNA ploidy status and LNM (Stalberg et al., 2017), and it should not be included in the preoperative decision on lymphadenectomy (Colombo et al., 2016, Stalberg et al., 2017).
CTNNB1

The catenin beta 1 (CTNNB1) gene encodes the protein β-catenin, a component of the E-cadherin–catenin unit, which plays an important role in normal cell and tissue architecture. Mutations in CTNNB1 cause accumulation of β-catenin in the cell nucleus, inducing gene transduction via the canonical Wnt pathway (Liu et al., 2002). CTNNB1 acts as a surrogate marker for activated Wnt/β-catenin signaling and appears in up to 47% of EEC and 3% of SEC (Matias-Guiu, Prat, 2013). Nuclear localization of β-catenin assessed by immunohistochemistry detected 85% of ECs with mutated CTNNB1 gene in a study of 345 EC patients (G. Kim et al., 2018). IHC could serve as an initial screen, with CTNNB1 sequencing employed when nuclear localization of β-catenin is absent (G. Kim et al., 2018).

CTNNB1 mutation is associated with low-grade endometrioid histology, younger age, < 50% myometrial invasion and absence of lymphovascular space invasion; these clinicopathological characteristics are traditionally associated with good prognosis (Kurnit et al., 2017). Interestingly, CTNNB1 mutation is also associated with high risk for recurrence and decreased recurrence-free survival in EEC (Kurnit et al., 2017, Myers et al., 2014, Stelloo et al., 2016). CTNNB1 mutations may impel VEGF activation and therefore respond to bevacizumab (Aghajanian et al., 2018, Arend et al., 2018).

TP53

A key tumor suppressor gene, tumor protein 53 (TP53) is mutated in over 50% of human cancers (Muller, Vousden, 2013, Sigal, Rotter, 2000). TP53 mutation is found in approximately 15% of EECs and 88% of SECs (Schultheis et al., 2016). SEC acquires TP53 alterations at onset, whereas in EEC, aberrant P53 expression is a late event (Hoang et al., 2013).

P53 IHC works as a precise surrogate, reflecting the underlying TP53 mutation status of EC (Kobel et al., 2018). Abnormal P53 is an independent prognostic marker for worse recurrence-free survival in grade 3 EEC (Bosse et al., 2018) and other high-risk EC (Stelloo et al., 2015, Talhouk, McAlpine, 2016).
L1CAM

The L1 cell adhesion molecule (L1CAM) is a membrane glycoprotein that plays a significant part in neurogenesis by regulating cell adhesion and migration (Kiefel et al., 2012, Rathjen, Schachner, 1984). L1CAM expression in EC tumor cells endorses disease progression by the enhancement of cell motility, invasion and metastasis (Kiefel et al., 2012, Weinspach et al., 2014).

An L1CAM threshold of > 10% staining has been demonstrated in 7–18% of lower-risk early-stage EC tumors (Bosse et al., 2014a, Fogel et al., 2003, Zeimet et al., 2013), and it is associated with an elevated risk of recurrences, especially distant relapses as well as poorer survival overall (Bosse et al., 2014b, Pasanen et al., 2016, Zeimet et al., 2013). An L1CAM threshold > 50% correlates with an elevated rate of distant metastases in high-risk EC (Van Gool et al., 2016).

HER2

The ERBB2 gene codes the human epidermal growth factor receptor 2 (HER2) attached to the cell membrane. HER2 activation leads to the transcription of genes promoting oncogenic transformation through cell proliferation, angiogenesis, survival and metastasis (Yarden, Sliwkowski, 2001).

HER2 is overexpressed particularly in NEEC and is associated with belligerent tumors and poor survival (Halle et al., 2018, Jones et al., 2015). In a phase II trial comparing paclitaxel-carboplatin with and without HER2 targeting trastuzumab in patients with advanced or recurrent uterine serous carcinoma overexpressing HER2, a significant decrease in the risk of progression was found with trastuzumab compared to paclitaxel-carboplatin alone (Fader et al., 2018).

ER/PR

The expression of estrogen receptor (ER) and progesterone receptor (PR) is common in the estrogen-dependent EEC and is found in 20–50% of SEC (Mhawech-Fauceglia et al., 2013, Togami et al., 2012) and 10% of clear cell carcinomas (Togami et al., 2012). Loss of these markers is typical in EC tumorigenesis and associates with a reduced disease-free and overall survival (Huvila et al., 2013, Trovik et al., 2013, van der Putten et al., 2018, Zhang et al., 2015).
2.6 TREATMENT

2.6.1 SURGERY

The goal of EC surgery is definitive treatment, or if surgery alone is not curative, it should abolish gross disease and stage the patient for a decision on postoperative adjuvant treatment. The mainstay surgery consists of total hysterectomy, BSO and peritoneal washing. Additional omentectomy is performed in cases with SEC. SLN mapping or comprehensive lymphadenectomy is carried out based on tailored risk assessment (Amant et al., 2018, Colombo et al., 2016)

Complications

Surgical site infection (SSI), an infection at the site of surgery within 30 days after an operation is the most frequent complication of gynecologic procedures. SSIs are classified as incisional or organ/space. Incisional SSIs are further divided into those involving only skin and subcutaneous tissue (superficial incisional) and those involving the deeper soft tissues of the incision such as muscle or fascia (Horan, Andrus & Dudeck, 2008).

The total rate of infectious complications was 7.7% after abdominal hysterectomy performed for benign causes in a Finnish prospective national study (Brummer et al., 2011). In a study on solely EC patients published by Bakkum-Gamez et al. the overall risk of SSI was 9.9%. Obesity was the most significant perioperative predictor of superficial incisional SSI, while surgical intricacy and older age were predictors for organ/space SSI (Bakkum-Gamez et al., 2013). The higher SSI risk profile of EC patients is based on malignancy per se, presumably via the immunology of carcinogenesis (Kadija et al., 2012). Additionally, the EC patients tend to be elderly and overexpress in obesity and diabetes, which are factors associated with SSI (Erekson et al., 2011, Brummer et al., 2011, Ko et al., 2014, Korol et al., 2013). More extensive cancer surgery causes an even longer operative time, both risks for SSI. Smoking and bad nutritional status increase the risk of SSI, and there are several preventive strategies used pre-, per- and postoperatively to minimize SSI risk (ACOG Practice Bulletin, 2018, Steiner, Strand, 2017).

Excessive surgery with lymphadenectomy predisposes patients to nerve and vessel damage, lymphocysts and lymphedema that depress the quality of life and expand the management costs (Dowdy et al., 2012, Frost et al., 2017, Yost et al., 2014).
Modalities

A laparoscopic approach (traditional or robotic) in surgical staging has become standard as it has proven to be as safe and efficient as laparotomy along with decreased operation–associated complications (Mourits et al., 2010, Obermair et al., 2012, Walker et al., 2009) and equal oncologic prognosis (Galaal et al., 2012, Janda et al., 2017, Koskas et al., 2016, Walker et al., 2012). Contraindication for laparoscopic surgery remains an enlarged uterus requiring morcellation and anesthesiological inconstancy to maintain a deep Trendelenburg position.

Vaginal hysterectomy is the safest, fastest and most economical approach when compared to other surgical techniques for benign hysterectomy (Makinen et al., 2013, Warren et al., 2009) and is the preferred route in benign gynecologic surgery (Committee on Gynecologic Practice, 2017). In EC patients, vaginal hysterectomy with or without traditional laparoscopic lymphadenectomy results in similar surgical and oncological outcomes and lower expenses than robotic modality (Nitschmann et al., 2017). A vaginal approach may be used in patients with redundant co-morbidities or low-risk disease (Amant et al., 2018, Nitschmann et al., 2017).

Robotic surgery has a faster learning curve and ergonomy for surgeons compared to traditional laparoscopy (Gala et al., 2014). For morbidly obese women, robotic surgery also shows a lower rate of conversions to laparotomy, a greater rate of performed pelvic lymphadenectomy and a shorter hospital stay than traditional laparoscopy. The oncologic outcomes are equal to the traditional route in obese women (Corrado et al., 2018). Overall, the two laparoscopic routes show similar perioperative morbidity and oncologic outcomes, but the robotic approach stands for substantially greater direct hospital costs (Cardenas-Goicoechea et al., 2014, Maenpaa et al., 2016, Turunen et al., 2013, Vuorinen et al., 2017, Wright et al., 2012).

Long-term health-related quality of life (HRQoL) six months after EC operation appeared similar in patients who had undergone laparoscopy and laparotomy in the LAP2 trial (Kornblith et al., 2009), while the results favored laparoscopy in research by Zullo et al. (Zullo et al., 2005). A new randomized trial reported no difference in the quality of life in relation to surgical modality at 12 months (Salehi et al., 2018).
2.6.2 ADJUVANT THERAPY

The prevailing challenge for treatment is to bypass adjuvant therapy and its associated toxicities for patients at low risk of recurrence, while simultaneously boosting therapy and amplifying cure rates for patients at high risk of recurrence.

The current tailored approach using postoperative risk assessment designed by ESMO-ESGO-ESTRO and complied by FIN-GOG, shown in Table 5, is adopted to avoid unnecessary over- and undertreatment and to ensure a fair quality of life (Colombo et al., 2016, FIN-GOG, 2018). Chemotherapy and radiotherapy have side effects such as emesis, gastrointestinal and genitourinary problems, lymphedema and hair loss (Joly et al., 2014, Nout et al., 2010, Paulsson et al., 2009).

Three large randomized trials, ASTEC, GOG#99 and PORTEC-1, showed adjuvant pelvic radiation therapy to be beneficial only for high-risk patients. A considerable reduction in the rates of vaginal and pelvic recurrence after external beam radiation therapy was noticed, yet without an added survival benefit. On the contrary, it increased the risk of overall morbidity (ASTEC/EN.5 Study Group et al., 2009, Creutzberg et al., 2000, Keys et al., 2004). The PORTEC-2 trial showed that vaginal brachytherapy had similar vaginal control rates compared with external beam radiotherapy but with substantially less adverse effects and a significantly better quality of life (Nout et al., 2010, Wortman et al., 2018). In a Danish cohort study, the exclusion of radiotherapy caused elevated recurrence rates without stirring survival rates in high and intermediate-risk patients; 22% of the recurrences occurred in intermediate-risk patients, of which 15% were locoregional (Ortoft, Hansen & Bertelsen, 2013). Adjuvant radiation therapy does not improve survival in low-risk patients. However, vaginal, pelvic and distant recurrences in low and intermediate risk groups do occur.

Adjuvant radiotherapy only and chemotherapy only have demonstrated similar effects on overall or relapse-free survival in EC patients (Maggi et al., 2006, Susumu et al., 2008). In a pooled analysis of 2 randomized trials studying the therapeutic value of combining adjuvant platinum-based chemotherapy with WPRT in patients with risk factors (grade 3 or deep invasion or adverse histologies), a notable 9% improvement in progression-free survival (69% vs. 78% at 5 years) was found with the addition of chemotherapy to WPRT, as well as a trend for a 7% improvement in 5-year overall survival (Hogberg et al., 2010).

EC is fairly chemo-sensitive. Platinum-based drugs, taxanes, and anthracyclines display the best response. Paclitaxel and carboplatin is a standard initial
combination therapy for advanced EC (Aghajanian et al., 2018, Miller et al., 2012).

**Table 5.** Postoperative profiling of endometrial cancer

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Stage and grade</th>
<th>Adjuvant therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LND not performed</td>
<td>LND performed</td>
</tr>
<tr>
<td><strong>Low risk</strong></td>
<td>St. IA G1-2, LVI-</td>
<td>None</td>
</tr>
<tr>
<td><strong>Intermediate risk</strong></td>
<td>St. IA, G3;</td>
<td>+/- Brachytherapy *</td>
</tr>
<tr>
<td></td>
<td>St. IB G1-2, LVI-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>St. 1 G1-2, LVI +;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>St. IA G3</td>
<td>WPRT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G3 and LVI-→BT</td>
</tr>
<tr>
<td><strong>High-intermediate risk</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>St. IB, G3</td>
<td>WPRT</td>
</tr>
<tr>
<td></td>
<td>St. II G1-3</td>
<td>WPRT</td>
</tr>
<tr>
<td></td>
<td>Nonendometrioid</td>
<td>Based on reoperation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reoperation not possible-&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>chemotherapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+/- BT/WPRT</td>
</tr>
<tr>
<td><strong>High-risk</strong></td>
<td>St. III, no residual</td>
<td>Chemotherapy +WPRT</td>
</tr>
<tr>
<td><strong>Advanced</strong></td>
<td>St. III residual +;</td>
<td>Case-specific</td>
</tr>
<tr>
<td></td>
<td>St. IVA</td>
<td></td>
</tr>
<tr>
<td><strong>Metastatic</strong></td>
<td>St. IVB</td>
<td>Case-specific</td>
</tr>
</tbody>
</table>

*+ Especially if ≥ 60 years old, BT = Brachytherapy, WPRT=whole body pelvic radiation therapy, LVI=lymphovascular space invasion, LND=lymphadenectomy
Adopted from ESMO-ESGO-ESTRO guidelines (Colombo et al., 2016) and FIN-GOG guidelines (FIN-GOG, 2018).
Molecular risk assessment has the potential to guide adjuvant therapy in integration with clinicopathological risk assessment. The ongoing PORTEC-4 trial explores the application of combined clinicopathologic, immunohistochemical, and molecular markers to determine the use of adjuvant vaginal brachytherapy or observation, preserving WPRT only for patients with high-risk factors.

2.6.3 TREATMENT OF INOPERABLE PATIENTS

Medically inoperable patients, such as the cardiopulmonary critically ill or the morbidly obese, can be treated with brachytherapy or, when high-risk factors present, with combined brachytherapy and WPRT (Podzielinski et al., 2012, van der Steen-Banasik et al., 2016, Amant et al., 2018). High-dose progestins, medroxyprogesterone acetate (MPA) 250 mg or megestrol acetate (MA) 160 mg may also be used alone or in combination with an intrauterine levonorgestrel-releasing device (LNG-IUD) (Amant et al., 2018, FIN-GOG, 2018).

2.6.4 FERTILITY SPARING TREATMENT

Fertility-preserving treatment can only be recommended in local noninvasive grade 1 EEC (Koskas et al., 2014). Promising results have been reported also from small studies of local G2 EEC treated conservatively (Hwang et al., 2017), but a larger retrospective analysis shows an elevated risk with grade 2–3 EEC (Gonthier, Trefoux-Bourdet & Koskas, 2017). An informative discussion with the patient of the non-standard treatment and consensus with careful follow up and later hysterectomy is crucial.

Diagnosis is made by hysteroscopy, dilatation and curettage and MRI. Medical treatment is based on oral progestins such as MA (160–320 mg/d) or MPA (400–600 mg/d). LNG-IUD can be added to peroral treatment or in combination with GnRH analogs (Wei et al., 2017). Evaluation of response must be performed by hysteroscopy plus curettage and MRI at 6 months. Complete response rate at 6 months is around 75%. Later on, 30–40% of them relapse (Gallos et al., 2012, Park et al., 2013).

The infertility clinic plans pregnancies for complete responders as soon as possible, and hysterectomy is committed shortly after childbearing.
2.6.5 TREATMENT OF RECURRENT DISEASE

An estimated 13% of all ECs diagnosed relapse. Of these, 80% occur within three years and over 50% are symptomatic (Sartori et al., 2010). Except for localized vaginal metastases, tumor recurrence is associated with a median expected survival of only 7–12 months (Fung-Kee-Fung et al., 2006, Sartori et al., 2010, Sorbe, Juresta & Ahlin, 2014). Typical sites of recurrent disease are pelvic and para-aortic lymph nodes, vagina, peritoneum and lungs. Vaginal recurrence is correlated with CSI, whereas extravaginal pelvic recurrence is correlated with deep myometrial and parametrial invasion and pelvic lymph nodes (Aalders, Abeler & Kolstad, 1984). More atypical sites such as the intra-abdominal organs, bones, brain, abdominal wall and muscle also occur. The most common intra-abdominal solid organ involved is the liver (7%) (Aalders, Abeler & Kolstad, 1984, Sohaib et al., 2007).

Location of the recurrence, whether isolated or multi-localized, and the primary treatment are the factors that decide which therapeutic strategy is used. In vaginal or central pelvic recurrence, a combination of WPRT and brachytherapy is suitable if radiation was not given in the first line. Large recurrency is evaluated for surgical removal followed by radiotherapy. Surgery is an option if cytoreduction with no macroscopic residual disease is feasible (Amant et al., 2018). Pelvic exenteration in well-selected patients with central recurrence and no distant metastasis may be an option. Palliative surgery is performed in selective patients to relieve symptoms like bowel obstruction or bleeding.

Platinum-based chemotherapy is recommended in recurrent disease, not suitable for or in combination with surgery or radiotherapy (Amant et al., 2018, Akram, Maseelall & Fanning, 2005). Doxorubicin, liposomal doxorubicin, and topotecan may be used in further therapy lines. According to NCCN guidelines, pembrolizumab is indicated for patients with MSI-H or MMR-tumors that have progressed following prior cytotoxic chemotherapy (Koh et al., 2018).

Hormonal therapy may be indicated for patients with advanced or recurrent EEC. Response rates (RR) of MPA were 37%, 23% and 9% for grade 1, grade 2 and grade 3 tumors, respectively (J. T. Thigpen et al., 1999). ER- and PR-positive disease show 25% and 37% response rates, respectively, but ER- and PR-negative disease show only a 7-8% response (Decruze, Green, 2007, J. T. Thigpen et al., 1999). The progestins, MPA 200 mg or MA 160 mg, have been recommended (Colombo et al., 2016, J. T. Thigpen et al., 1999). A biopsy of recurrent disease is advisable since the tumors may change their hormone receptor expressions along the way (Tangen et al., 2014).
Single therapy with tamoxifen, aromatase inhibitors and fulvestrant all demonstrate only a modest response rate of around 10% in phase II trials. However, letrozole 2.5mg x1 or tamoxifen 40mg x1 can be worth a try in advanced EC (Colombo et al., 2016, T. Thigpen et al., 2001).
This thesis was designed to evolve a clinically applicable and reliable approach for the risk assessment of advanced disease in EC and to gauge the EC patient’s risk for surgery-related mortality and disease-related long-term morbidity.

The specific objectives of the study were:

1. To develop a clinically convenient and reliable risk-scoring model that could predict the likelihood of LNM and distant metastasis in EC.

2. To evaluate the developed risk-scoring system by comparing its performance characteristics with two other models in predicting lymphatic dissemination in EC.

3. To investigate the correlation of predictors of advanced disease and/or poor outcome with the manifestation of tumor relapses in different anatomical sites in patients with stage I–II EEC.

4. To determine the risk factors of surgical site infection after EC operation.
4 MATERIAL AND METHODS

4.1 SUBJECTS

The study design is a retrospective cohort study based on prospectively recorded data. The cohort in study I consists of all the consecutive 1166 women who were surgically treated for EC between January 1, 2007, and December 31, 2013, at the Department of Obstetrics and Gynecology, Helsinki University Hospital. The cohort in study II consists of all 1052 of the study I cohort women with endometrioid-type tumor stage I–III. The study III cohort includes all 929 stage I and stage II EEC patients from the study I cohort. Finally, the study IV cohort comprises 1164 study I women who were treated with hysterectomy. The cohorts are shown in Figure 2, and the clinicopathological data are shown in Table 6. The study was approved by the Institutional Review Board and the National Supervisory Authority for Welfare and Health (135/13/03/03/2013).

Figure 2. Formation of the study cohorts.
4.2 SURGERY

Traditional laparoscopy was carried out for 66.7% (778/1166), robotic laparoscopy for 6.2% (72/1166), vaginal for 0.9% (11/1166) and laparotomy for 26.2% (305/1166) of patients. Conversions, a rate of 5.6% (51/912 for the minimally invasive group only) were included in the laparotomy rate. Pelvic and para-aortic lymphadenectomy were suggested for patients at high risk for disseminated disease, estimated as those with MI $\geq$ 50% grade 1–2 EEC, grade 3 EEC and NEEC. At the beginning of the study period, MI was evaluated by gross visual inspection and pelvic lymphadenectomy only was advocated for patients with superficial grade 1–2 EEC.

From January 2012 onward, lymphadenectomy was omitted in grade 1–2 EEC with MI < 50% estimated with MRI. Patient comorbidities and frailty influenced the surgeons’ decisions on lymphadenectomy and the extent of the procedure. The total lymphadenectomy rate was 64.8% (755/1166). SLN mapping was assessed in 1.2% (14/1166). Pelvic or pelvic-aortic lymphadenectomy was carried out for 65.2% (497/762) of the patients with stage I–IIIB cancer and Mayo high-risk criteria in the final pathologic evaluation.
Table 6. Clinicopathologic data (n=1166).

<table>
<thead>
<tr>
<th>Clinicopathologic data</th>
<th>n=1166</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean ± SD)</td>
<td>67.5 ± 10.5</td>
</tr>
<tr>
<td>BMI (kg/m2) (mean ± SD)</td>
<td>28.6 ± 6.3</td>
</tr>
<tr>
<td>Pelvic lymphadenectomy (number, percent)</td>
<td>580 (49.7%)</td>
</tr>
<tr>
<td>Pelvic-aortic lymphadenectomy (number, percent)</td>
<td>175 (15.0%)</td>
</tr>
<tr>
<td>Lymph node yield, pelvic lymphadenectomy (mean ± SD)</td>
<td>15.2 ± 8.1</td>
</tr>
<tr>
<td>Lymph node yield, pelvic-aortic lymphadenectomy (mean ± SD)</td>
<td>26.8 ± 11.1</td>
</tr>
<tr>
<td>Surgical modality (number, percent)</td>
<td></td>
</tr>
<tr>
<td>Traditional laparoscopy</td>
<td>778 (66.7%)</td>
</tr>
<tr>
<td>Robotic laparoscopy</td>
<td>72 (6.2%)</td>
</tr>
<tr>
<td>Laparotomy</td>
<td>305 (26.2%)</td>
</tr>
<tr>
<td>Vaginal hysterectomy</td>
<td>11 (0.9%)</td>
</tr>
<tr>
<td>Conversions</td>
<td>51 (5.6%)</td>
</tr>
<tr>
<td>Histology (number, percent)</td>
<td></td>
</tr>
<tr>
<td>Endometrioid carcinoma</td>
<td>1070 (91.8%)</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>41 (3.5%)</td>
</tr>
<tr>
<td>Serous carcinoma</td>
<td>34 (2.9%)</td>
</tr>
<tr>
<td>Undifferentiated carcinoma</td>
<td>20 (1.7%)</td>
</tr>
<tr>
<td>Neuroendocrine carcinoma</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>Grade (number, percent) (For endometrioid only, n = 1070)</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>645 (60.3%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>268 (25.0%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>157 (14.7%)</td>
</tr>
<tr>
<td>FIGO stage (number, percent)</td>
<td></td>
</tr>
<tr>
<td>IA</td>
<td>679 (%8.2%)</td>
</tr>
<tr>
<td>IB</td>
<td>226 (19.4%)</td>
</tr>
<tr>
<td>II</td>
<td>66 (5.7%)</td>
</tr>
<tr>
<td>IIIA</td>
<td>54 (4.6%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>11 (0.9%)</td>
</tr>
<tr>
<td>IIIC1</td>
<td>56 (4.8%)</td>
</tr>
<tr>
<td>IIIC2</td>
<td>33 (2.8%)</td>
</tr>
<tr>
<td>IVA</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>IVB</td>
<td>41 (3.5%)</td>
</tr>
</tbody>
</table>

1 Number of cases 1165 (BMI of 1 patient unknown); 2 Number of cases 571 (lymph node yield was not available for 9 patients); 3 Number of cases 173 (lymph node yield was not available for 2 patients); 4 Including 19 carcinosarcomas
4.3 LABORATORY ANALYSES

The most recent preoperative complete blood count was analyzed by photometric measurement (hemoglobin) along with electrical impedance technology and flow cytometry (cells). Blood count variables were not available for one patient. Pretreatment serum CA-125 concentration was quantitated with a chemiluminescent microparticle immunoassay on the Abbott Architect 2000i Analyzer (Abbott Diagnostics, Abbott Park, IL, USA). CA-125 values were not available for 122 patients.

4.4 PREOPERATIVE HISTOLOGY

Preoperative endometrial histology was analyzed in tissue samples collected mostly by endometrial biopsy. Uterine curettage was implemented when a biopsy was insufficient for diagnosis or when it failed. An endometrial sample was not taken or was nondiagnostic in 26 patients. Two samples for which the original pathology review did not clearly differentiate between low-risk histology and high-risk histology were re-evaluated.

4.5 INTRAOPERATIVE TUMOR SIZE

Tumor size was approximated from bisected uteri intraoperatively by the surgeon and/or after formaldehyde fixation by the pathologist. Tumor diameter was distinguished as the largest dimension of the tumor. If more than 1 lesion was present, the lesion with the largest diameter was considered. In dubious cases, i.e., when the endometrium contained a polyp or was diffusely abnormal in gross appearance, tumor size was based on the macroscopic appearance without reference to the histological evaluation. Primary tumor diameter was not available for 63 patients.

4.6 FOLLOW-UP PROTOCOL

After first-line treatment, 881 patients (94.8%) were followed up at Helsinki University Hospital or at one of the affiliated hospitals of the Helsinki and Uusimaa district. Forty-five patients (4.8%) were followed elsewhere in Finland, and 3 patients (0.3%) at various medical units. The standard protocol included a check-up at 3- to 4-month intervals for 1 year, and every 6 to 12 months thereafter for at least 3 years. The appointments involved physical examinations, completed by imaging studies when indispensable, depending on symptoms and findings. The time of follow-up was determined from the date of surgery until the last
patient check from an electronic patient data system or by contacting the treating physician at the referring hospitals. Lacking data were obtained from Statistics Finland.

4.7 DEFINITION OF RELAPSE

The imaging modality of choice in diagnosing a relapse was mostly whole-body CT. At the first diagnosis of cancer recurrence the relapses were categorized according to their anatomical site:

1. Isolated vaginal relapses
2. Pelvic relapses, including those with concomitant vaginal relapse; pelvic and para-aortic lymph node relapses were included in this category (also when extended to the groin)
3. Intra-abdominal relapses beyond the pelvis, including those with a concomitant vaginal or pelvic relapse
4. Extra-abdominal relapses beyond the pelvis, including those with a concomitant relapse in any other site

4.8 DEFINITION OF SURGICAL SITE INFECTION

SSI was defined as an infection at the site of surgery within 30 days after hysterectomy (Mangram et al., 1999). The SSIs were classified as incisional and organ/space infections, as shown in Table 7. Data on SSIs were collected from institutional medical records. The primary physicians at the referring institutions were contacted to ascertain information on SSIs if follow-up data were lacking. Postoperative infections other than SSI, such as urinary tract infection or pneumonia, were not considered in this study.
**Table 7.** Criteria for defining SSI. Adopted from Mangram et al. (Mangram et al., 1999).

<table>
<thead>
<tr>
<th><strong>Superficial incisional surgical site infection</strong>&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection involves only skin or subcutaneous tissue of the incision and &lt;i&gt;at least one&lt;/i&gt; of the following:</td>
<td></td>
</tr>
<tr>
<td>1. Purulent drainage, with or without laboratory confirmation, from the superficial incision.</td>
<td></td>
</tr>
<tr>
<td>2. Organisms isolated from aseptically obtained culture of fluid or tissue from the superficial incision.</td>
<td></td>
</tr>
<tr>
<td>3. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat and superficial incision is deliberately opened by surgeon, unless incision is culture-negative.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Deep incisional surgical site infection</strong>&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection involves deep soft tissues (e.g. fascial and muscle layers) of the incision and &lt;i&gt;at least one&lt;/i&gt; of the following:</td>
<td></td>
</tr>
<tr>
<td>1. Purulent drainage from the deep incision but not from the organ/space component of the surgical site.</td>
<td></td>
</tr>
<tr>
<td>2. A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (&gt;38°C), localized pain, or tenderness, unless site is culture-negative.</td>
<td></td>
</tr>
<tr>
<td>3. An abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Organ/space surgical site infection</strong>&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection involves any part of the anatomy (e.g. organs or spaces), other than the incision, which was opened or manipulated during an operation and &lt;i&gt;at least one&lt;/i&gt; of the following:</td>
<td></td>
</tr>
<tr>
<td>1. Purulent drainage from a drain that is placed through a stab wound into the organ/space.</td>
<td></td>
</tr>
<tr>
<td>2. Organisms isolated from an aseptically obtained culture or fluid or tissue in the organ/space.</td>
<td></td>
</tr>
<tr>
<td>3. An abscess or other evidence of infection involving the organ/space that is found on direct examination, during reoperation, or by histopathologic or radiologic examination.</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Diagnosis by the surgeon or attending physician also meets the criteria for each type of surgical site infection.
4.9 STATISTICAL ANALYSES

In study I, Pearson’s $\chi^2$ analyses were used to compute odds ratios along with 95% confidence intervals for the associations between each risk factor and lymph node and distant metastasis (stage IIIC–IV carcinoma) in the cohort. Factors identified as potential risk factors in unadjusted analyses ($P < 0.05$) were used to create a logistic regression model with lymph node and distant metastasis as the dependent variable. Patients with available data for all potential risk factors were included in the regression model ($n = 969$). An internal sensitivity analysis of the regression model was performed by bootstrapping (Dwivedi, Mallawaarachchi & Alvarado, 2017) with 1000 repetitions to obtain unbiased estimates of the standard errors and $P$ values of the model. Statistically significant odds ratios in the multivariable model were rounded to the nearest integer. All the rounded values were even numbers, which allowed them to be divided by a factor of 2 to then be used as the estimated weights for the risk factors. A weighted sum of risk factors was calculated for each patient to generate a set of risk score points that might determine the predictive values probability of stage IIIC–IV carcinoma. Sensitivity, specificity, and positive and negative (with 95% confidence intervals) were calculated for each summed value of risk score points. To evaluate the capability of the model to predict lymph node involvement only, diagnostic indices were calculated separately for patients who underwent pelvic or pelvic-aortic lymphadenectomy in a dataset without stage IV carcinomas ($n = 629$). The proportions of various uterine risk factors in patient subgroups with either a low or high risk for advanced disease were compared by Fisher’s exact test.

In study II, the area under the receiver operating characteristic curve was built for each model. The areas under curve were compared with the 2-tailed receiver operating characteristic curve area comparison test. The chi-square and Fisher’s exact test were used to compare categorical variables. Disease-specific survival, defined as the time from the date of surgery to death from endometrial cancer, was estimated using the Kaplan-Meier method. Differences between groups were compared using the log-rank test. The joint effect of 2 factors on survival was assessed using multivariable Cox regression analysis. The median follow-up time was 55 months (range 1–108). Follow-up time was calculated from the date of surgery until the last patient status check from an institutional electronic patient data system. This system combines medical records from all specialties in medicine and receives weekly updates on patient deaths from the Population Register Center of Finland. Lacking follow-up data were derived from Statistics Finland or completed by contacting the treating physician at the referring hospitals.
In study III, Pearson’s χ² or Fisher’s exact test was used to compute odds ratios (ORs) along with 95% confidence intervals (CIs) for the associations between each risk factor and tumor relapses in different sites. Factors identified as potential risk factors in unadjusted analyses (P < 0.10) were used to create logistic regression models, with site-specific relapses as the dependent variables. Multiple imputation was used to account for missing data. All the studied variables were used in the imputation models. Pooled data of 5 drawn imputations were analyzed. Univariate Cox regression analysis was used to assess the importance of the anatomical location of the disease relapse in disease-specific survival. Disease-specific survival was defined as the time from surgery to death from endometrial cancer.

In study IV, comparisons between women with and without SSI were evaluated with a chi-squared test or Fisher’s exact test for categorical variables and with the Mann–Whitney U-test for continuous variables. Factors identified as potential risk factors in unadjusted analyses (p < 0.15) were used to create logistic regression models with incisional and organ/space SSIs as the dependent variables. Women with available data for all potential risk factors were included in the regression models.

Statistical significance was set at p < 0.05 in all studies. Data were analyzed using IBM SPSS version 22 or 24 software (IBM Corp., Armonk, NY, USA).
5 RESULTS

5.1 MODEL FOR PREDICTION OF LYMPHATIC DISSEMINATION

In the first study, a combined preoperative and intraoperative risk score utilizing previously recognized risk factors of lymph node and distant metastases was created. The optimal tumor size for predicting the risk for advanced disease was chosen by evaluating the capability of primary tumor diameters of ≥ 2 cm and ≥ 3 cm to function as surrogates for a ≥ 50% myometrial invasion. Areas under curve for cut-off values of 2 cm and 3 cm were 0.645 and 0.701, respectively, in predicting deep MI (P = 0.024 by receiver operating characteristic curve area comparison test). Thus, a primary tumor diameter of 3 cm was chosen as the cut-off value for this study.

Of the selected putative risk factors, all the potential risk factors with the exception of age were associated with an increased risk for stage IIIC–IV disease in unadjusted analyses: normal weight (BMI < 25 kg/m2), anemia (hemoglobin < 117 g/l), leukocytosis (leukocytes > 8.7 x 10⁹/l), thrombocytosis (thrombocytes > 360 x 10⁹/l), elevated CA-125 (> 35 U/ml) and high-risk histology (i.e., grade 3 endometrioid, clear cell, serous, undifferentiated, neuroendocrine) and large tumor.

Thrombocytosis, elevated serum CA-125, preoperative high-risk histology and tumor size ≥ 3 cm remained significant predictors for advanced disease in the logistic regression analysis before and after bootstrapping. Statistically significant odds ratios were rounded to the nearest integer and divided by a factor of 2, therefore giving thrombocytosis 1 point, G3/nonendometrioid histology 2 points, tumor diameter > 3 cm 2 points and CA-125 > 35U/ml 3 points.

The total risk score points ranged from 0 to 8 for individual patients. With a cut-off of 1 point, stratifying 66.3% of patients to surgical staging, the model predicted stage IIIIC–IV carcinomas with a sensitivity of 100%, specificity of 38.0%, positive predictive value (PPV) of 17.1% and negative predictive value of 100%. With the same cut-off, the corresponding values were 100%, 34.7%, 16.5% and 100% in predicting stage IIIC carcinomas in a subgroup that underwent lymphadenectomy and had stage I–IIIC disease.
results

5.2 COMPARISON OF THREE RISK MODELS

Indicating a fair to good discrimination power for all models, the areas under curve in predicting lymphatic dissemination were 0.781, 0.830 and 0.829 for the Mayo, Helsinki ($P = 0.285$ vs. Mayo) and Milwaukee ($P = 0.292$ vs. Mayo) models, respectively. These findings were consistent after the removal of patients not subjected to lymphadenectomy. With the cut-off set at low-intermediate risk for the Mayo and Milwaukee models and with 1 risk score point for the Helsinki model, sensitivity was highest for the Helsinki model and specificity highest for the Milwaukee model, with the sensitivity/specificity being 94.4%/30.2%, 100%/40.6% and 91.0%/54.2% for the Mayo, Helsinki, and Milwaukee models, respectively. These findings remained when the analyses were limited to patients who underwent pelvic or pelvic-aortic LND.

The rates of false negatives and false positives were similar for all models. The lymphadenectomy rate decreased in the order of Mayo model (71.5%) > Helsinki model (62.4%) > Milwaukee model (48.8%). In patients with stage I cancer, disease-specific survival was better for those who satisfied low-risk criteria according to any of the models. In patients with stage II–III cancer, this difference in survival was significant only for the Milwaukee model. Both lymphatic dissemination and high-risk tumor features per the risk models were independent predictors of survival.

5.3 PREDICTION OF SITE-SPECIFIC RELAPSES

During a median follow-up time of 57 months (range 1–108 months), a relapse was diagnosed in 98 patients (10.5%). A vaginal relapse was diagnosed in 15 patients (1.6%), pelvic relapse in 27 patients (2.9%), intra-abdominal relapse beyond the pelvis in 27 patients (2.9%) and extra-abdominal relapse in 29 patients (3.1%). Twenty-eight relapses (28.6%) were asymptomatic when diagnosed by the planned follow-up, and 70 (71.4%) were revealed by clinical symptoms occurring during the interval of follow-up investigations. There were no associations with altered risk of vaginal or pelvic relapses in univariate analyses in any of the studied variables: poor tumor differentiation, MI 50% or greater, tumor size 3 cm or greater, LVSI, CSI, abnormal peritoneal cytology, old age, obesity and diabetes.

Poor differentiation, MI 50% or greater, tumor size 3 cm or greater and abnormal peritoneal cytology were associated with an increased risk of intra-abdominal relapses (ORs between 2.3 and 13). A tumor size of 3 cm or greater (OR 3.1) and abnormal peritoneal cytology (OR 16) predicted intra-abdominal relapses beyond
the pelvis in multivariate analysis, whereas poor differentiation (OR 2.9), MI \( \geq 50\% \) (OR 4.0) and abnormal peritoneal cytology (OR 27) predicted extra-abdominal relapses (Table 8). Peritoneal cytology was considered positive if adenocarcinoma cells were detected in the peritoneal washing, regardless of the number of cancer cells. Five cases that were positive because of a concomitant ovarian tumor were considered negative for endometrial cancer. Peritoneal cytology status was not available for 12 patients.

**Table 8.** Prediction of distant relapses by multivariate analyses in stage I–II endometrioid endometrial cancer

<table>
<thead>
<tr>
<th></th>
<th>Intra-abdominal Relapses Beyond the Pelvis*</th>
<th></th>
<th>Extra-abdominal Relapses†</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>( P )</td>
<td>OR (95% CI)</td>
<td>( P )</td>
</tr>
<tr>
<td>Tumor grade 3</td>
<td>2.3 (0.88–6.3)</td>
<td>0.089</td>
<td>2.9 (1.2–7.1)</td>
<td>0.023</td>
</tr>
<tr>
<td>Myometrial invasion ( \geq 50% )</td>
<td>1.3 (0.51–3.1)</td>
<td>0.626</td>
<td>4.0 (1.4–11)</td>
<td>0.008</td>
</tr>
<tr>
<td>Tumor size ( \geq 5 ) cm</td>
<td>3.1 (1.2–8.4)</td>
<td>0.025</td>
<td>1.6 (0.57–4.7)</td>
<td>0.355</td>
</tr>
<tr>
<td>Lymphovascular space invasion</td>
<td>—</td>
<td>—</td>
<td>1.2 (0.42–3.6)</td>
<td>0.698</td>
</tr>
<tr>
<td>Cervical stromal invasion</td>
<td>1.6 (0.49–5.3)</td>
<td>0.437</td>
<td>1.9 (0.67–5.5)</td>
<td>0.221</td>
</tr>
<tr>
<td>Positive peritoneal cytology</td>
<td>13 (2.8–61)</td>
<td>0.001</td>
<td>27 (5.9–120)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age &gt;77 y</td>
<td>—</td>
<td>—</td>
<td>2.1 (0.86–5.0)</td>
<td>0.105</td>
</tr>
<tr>
<td>Body mass index ( \geq 30 ) kg/m(^2)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Diabetes</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Factors identified as potential risk factors in unadjusted analyses (\( P < 0.010 \)) were included in the models. Missing data were replaced using multiple imputation.

†Patients with vaginal, pelvic, and extra-abdominal relapses were excluded.

A worse disease-specific survival was associated with intra-abdominal relapses beyond the pelvis and extra-abdominal relapses compared with vaginal relapses. Survival of patients with a pelvic relapse did not vary from that of patients with a vaginal recurrence (Table 9).
results

Table 9. Cox regression analysis for disease-related death in stage I–II endometrioid endometrial cancer with the site of relapse as a prognostic factor

<table>
<thead>
<tr>
<th>Site of Relapse</th>
<th>Hazard Ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagina</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Pelvis</td>
<td>3.8 (0.85-17)</td>
<td>0.080</td>
</tr>
<tr>
<td>Intra-abdominal beyond the pelvis</td>
<td>8.1 (1.9-35)</td>
<td>0.005</td>
</tr>
<tr>
<td>Extra-abdominal</td>
<td>9.4 (2.2-40)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Median follow-up time 34 months (range, 3-105 months)

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5.4 SURGICAL SITE INFECTIONS RELATED TO ENDOMETRIAL CARCINOMA

Ninety-four women (8.1%) were diagnosed with a surgical site infection. Twenty women (1.7%) had an incisional infection and 74 (6.4%) had an organ/space infection. The associations of 17 clinicopathologic and surgical variables were tested by univariate analyses. Variables that were identified as potential risk factors in univariate analyses ($p < 0.15$) were used in logistic regression models with incisional and organ/space infections as dependent variables. Obesity (body mass index $\geq 30$ kg/m$^2$), diabetes and long operative time (> 80th centile) were independently associated with a higher risk of incisional infection, whereas minimally invasive surgery was associated with a smaller risk. Smoking, conversion to laparotomy and lymphadenectomy were associated with a higher risk of organ/space infection.
EC, owing to its high prevalence, has been assiduously investigated for several decades. However, data for clear guidelines regarding surgical retroperitoneal staging has remained in conflict. Various large retrospective studies have shown the potential benefit of LND (Fotopoulou et al., 2010, Mariani et al., 2008). Meanwhile, according to two randomized trials involving a large group of patients, those with early-stage, low-grade EEC do not seem to profit from LND while they are predisposed to complications from the procedure (ASTEC study group et al., 2009, Benedetti Panici et al., 2008). The management of EC has changed to selective lymphadenectomy built on personalized risk assessment.

LNM can be predicted by high-risk features of the primary carcinoma, and these same factors affect the patient outcome further, both with LNM and in its absence (Barrena Medel et al., 2011, Kwon et al., 2009, Milgrom et al., 2014). Consequently, it is of eminent importance to detect the patients with poor prognosis as precisely and early as possible. The development of clinically feasible and accurate methods for the preoperative prediction of LNM and disease recurrence as well as evaluation of the existing models is key.

6 DISCUSSION

Lymph node involvement in EC is influenced by numerous uterine, demographic and biochemical factors. We were prompted to investigate the additive value of tumor size variable in predicting the risk for stage IIIC–IV EC by a risk model published previously by our research group. The model combines preoperative histology with demographic and biochemical risk factors (old age, normal weight, elevated CA-125) for advanced disease and poor outcome (Luomaranta, Leminen & Loukovaara, 2013).

The present study I population is large and reaches globally providing detailed information on different demographic- and disease-related parameters. This well-characterized cohort has enabled reasonable and reliable research conducted in the studies.

6.1 JOINT PREOPERATIVE AND PERIOPERATIVE PREDICTION OF ADVANCED DISEASE

Risk model

Lymph node involvement in EC is influenced by numerous uterine, demographic and biochemical factors. We were prompted to investigate the additive value of tumor size variable in predicting the risk for stage IIIC–IV EC by a risk model published previously by our research group. The model combines preoperative histology with demographic and biochemical risk factors (old age, normal weight, elevated CA-125) for advanced disease and poor outcome (Luomaranta, Leminen & Loukovaara, 2013).

The present study I population is large and reaches globally providing detailed information on different demographic- and disease-related parameters. This well-characterized cohort has enabled reasonable and reliable research conducted in the studies.
Proven by the logistic regression analysis and internal validation by a bootstrapping method, thrombocytosis, elevated CA-125, a tumor size of $\geq 3$ cm and preoperative high-risk histology (G3EEC and NEEC) are independent predictors of advanced EC. The total score, combining all risk factors with an independent effect on disease spread, ranged from 0 to 8, depending on existing risk factors of the patient. The performance of the score in detecting lymph node and distant metastasis was tested. A cut-off of 1 point was optimal because at this point, the total score had a good discriminatory power (AUC 0.852), good sensitivity (100%), specificity (38%) and high NPV (100%), minimizing the mislabeling of a patient with stage IIIC or stage IV disease.

The performance of the score to identify advanced disease was also tested in a subgroup from which stage IV carcinomas were excluded. The discriminatory power did not significantly differ (sensitivity 100% and NPV 100%) from that of the original analysis, suggesting that the model predicts LNM in addition to distant metastasis.

The reliability of the risk score is not achieved at the expense of a high lymphadenectomy rate. It stratified 66% of patients to surgical staging, which is in the same range as risk stratification based on a frozen section (73%) (Mariani et al., 2008). Despite the fact that deep MI was not used as a risk variable in this model, it was less common in patients with 0 risk score points compared with patients with $\geq 1$ point. This also applied to the presence of LVSI and CSI. Seemingly, various risk features are interrelated, as suggested by the good capability of a tumor size of $\geq 3$ cm to predict deep MI (AUC 0.701). The lack of these risk variables from our model did not compromise its reliability because regardless of the variable status, none of the patients with 0 risk score points was diagnosed with LNM.

Occasionally, appraisal of the tumor size from a bisected uterus is problematic since endometrial hyperplasia can compromise the whole uterine cavity, and benign polyps co-existing with cancer can also be misjudged as malignant. Such rare cases in this study were considered “large tumors” when this was consistent with the gross finding, without reference to the pathology report.

The advantage of the risk-scoring model is that it is highly reliable and simple, and it could be clinically widely applicable since its use does not require expensive examination modalities generally not available or dependent on high local expertise. The model is also favorable when making personalized decisions on lymphadenectomy, for example for patients with compelling surgical risks due to obesity or other comorbidities. Although the patients were treated in a single institution and therefore risk calculations should be cautiously generalized, it is
implausible that selection bias essentially influenced the results, as the stage distribution and demographics in our cohort were comparable to those recorded in other developed countries (Crosbie et al., 2012, Lewin, Wright, 2011, Werner et al., 2012).

**Comparison of the three risk models**

The primary surgery of an EC patient is customized based on individual risk assessment. The patients at low risk for lymphatic spread do not benefit from lymphadenectomy, which is associated with operative complications. An ideal risk-stratification method is accurate and is associated with a decent rate of lymphadenectomy, as well as being a predictor of patient survival. World-wide, probably the mostly used risk model was created by Mariani et al. (Mariani et al., 2000), further developed by Bogani et al. (Bogani et al., 2014) at the Mayo Clinic. Based on frozen section analysis, the Mayo criteria low-risk group consists of grade 1–2 endometrioid tumors with MI ≤ 50% and diameter of ≤ 2 cm and all noninvasive endometrioid tumors with any grade and size. Recently, Cox Bauer et al. at the Aurora Sinai Medical Center and St. Luke's Medical Center in Milwaukee, WI, presented a nomogram for endometrioid carcinomas that allows for an additional 20% of patients to be spared LND compared with the Mayo criteria. This Milwaukee low-risk group includes patients with a tumor depth of invasion ≤ 33% and a diameter ≤ 50 mm, regardless of grade (Cox Bauer et al., 2016).

In study II, it was of interest to compare the performance characteristics of the risk model developed in study I with a modified Mayo model and the Milwaukee model. As in the Milwaukee model, the cohort included endometrioid endometrial cancer patients and the analysis was based on findings from the final pathology. In the present study, the risk for LNM in Mayo low-risk patients with completed lymphadenectomy was 2.3%, which is in the same scope as in surgically staged Mayo criteria low-risk patients (1.4%) in a population-based analysis of patients with stage I or IIIC endometrioid endometrial cancer (Vargas et al., 2014). The proportion of low-risk patients was 28.5%, which is somewhat higher than the 21.1% reported by Vargas et al. (Vargas et al., 2014). This is partially explained by the fact that here, the Mayo criteria for low risk included noninvasive endometrioid carcinomas of any grade and size, and all the stages, whereas the population-based study Mayo criteria were defined as grade 1–2 endometrioid histology, MI < 50% and a tumor diameter of ≤ 2 cm (Vargas et al., 2014).
The proportion of low-risk cancers and their risk for LNM were 38.8% and 0%, respectively, in the original Milwaukee model (Cox Bauer et al., 2016). The higher proportion of low-risk Milwaukee patients, 51.2%, and the 2.0% LNM in study II did not diminish the accuracy of the model, as its area under curve in predicting LNM was 0.829, which is similar to the original cohort (0.829) (Cox Bauer et al., 2016). As anticipated, the findings of the Helsinki model were like the ones presented in study I.

The areas under curve in predicting LNM, as well as the rates of false positives and false negatives, were similar in all three tested models. The lowest lymphadenectomy rate was identified in the Milwaukee model. Institutions planning on implementing the Milwaukee model need to validate it with techniques that would be used for the evaluation of tumor size and depth of myometrial invasion, since both the Cox Bauer study (Cox Bauer et al., 2016) and this comparing study are based on the analysis of the final pathology.

A poorer disease-specific survival was found in patients with stage I cancer not satisfying low-risk criteria according to any of the models than in those with stage I low-risk cancer. This is in line with the findings that uterine risk factors predict the outcome of EC patients irrespective of nodal status (Barrena Medel et al., 2011, Kwon et al., 2009). This emphasizes the need for a precise risk-stratification model, predicting both LNM in the primary setting for an individual decision on LND and, concurrently, the risk of recurrence for the decision on adjuvant therapy.

### 6.2 SITE-SPECIFIC TUMOR RELAPSES

Peritoneal and hematogenous disseminations are frequently related to distant relapses intra-abdominally and extra-abdominally, whereas contiguous and lymphatic spread are linked in the progress of central relapses. Prior studies have shown many uterine risk factors to increase the risk of disease recurrence (Descamps et al., 1997, Grigsby et al., 1992, Mariani et al., 2001). Early-stage, high-risk EEC patients are in need of adjuvant therapy, which for now has constituted of a choice of WPRT and brachytherapy. However, it has been challenging to find a universal definition of true high-risk cases and indications of adjuvant therapy. The dilemma concerns a considerable group of patients; nearly 80% of all EC cases in Helsinki University Hospital (2007–2013, 929/1166 cases). Therefore, we investigated the association of uterine risk factors, peritoneal cytology status, and demographic factors with the existence of site-specific relapses in patients with stage I–II EEC.
During the median follow-up time of nearly 5 years, a relapse was diagnosed in 98 patients (10.5%), while the proportion of distant (intra-abdominal relapse beyond the pelvis and extra-abdominal relapse) relapses was rather high at 57.1%. None of the examined factors was associated with relapses in the vagina or pelvis in univariate analyses. In multivariate analyses, uterine risk factors and positive peritoneal cytology retained their significance as predictors of distant relapses. These findings result in valuable therapeutic implications. The relatively high rate of distant relapses in this study is consistent with several randomized trials that reported pelvic radiotherapy to improve locoregional control but not disease-specific or overall survival in stage I–II endometrial cancer (ASTEC/EN.5 Study Group et al., 2009, Keys et al., 2004, Nout et al., 2010). It seems possible that, because the uterine risk features were associated with distant relapses but not with locoregional relapses, the systemic protection provided by chemotherapy would be prognostically more important in early-stage high-risk cancers than the local control provided by radiotherapy.

The number of limitations concerning the study can be recognized, mostly due to its retrospective design. The possibility that the observed incidence and anatomical distribution of the relapses would have been different if their diagnostics had been performed in a standardized fashion, such as by routine imaging at regular intervals, cannot be excluded. On the other hand, the study setting represents a more real-life situation, where the extent of the diagnostics is directed by individual decisions depending on patient symptoms and status findings. This kind of personalized medical practice complies with the follow-up recommendations by the Society of Gynecologic Oncologists (Salani et al., 2011).

Preoperative imaging was used to exclude distant metastases, but the regional lymph node status was not thoroughly appraised as the rate of pelvic-aortic LND was scant at 10.0% (93/929). Contradictionally, the rate of pelvic LND or sentinel node mapping was 54.5% (506/929). Considering earlier reports of the low (1–3%) rate of skip metastases (Abu-Rustum et al., 2009, Kurman et al., 2014), it is presumable that the assessment of pelvic nodes alone adequately endorses node negativity.

### 6.3 SURGICAL SITE INFECTIONS

SSI is the most typical complication after surgery for a gynecologic reason. SSIs bring about tremendous healthcare costs and human suffering. Various studies have been addressed to investigate SSI after benign gynecologic surgery but only very few have examined SSI risks solely for EC patients. Bakkum-Gamez et al.
reported a 9.9% overall risk of SSI in a cohort of EC patients where most SSIs were of the superficial incisional type (65.4%), followed in frequency by organ/space (22.8%) and deep incisional (8.1%) (Bakkum-Gamez et al., 2013). In our study (study IV), the overall rate was 8.1%, and the distribution of SSI type was strikingly different from the Bakkum-Gamez et al. study. Here, organ/space infections comprised 78.7% of all SSIs, and a deep incisional SSI was diagnosed in one woman only (1.1%). Minimally invasive surgery was associated with a smaller risk of incisional SSI but not of organ/space SSI. Thus, the different proportions of incisional and organ/space SSIs could be explained by route of hysterectomy. This is supported by a recent study where the American College of Surgeons’ National Surgical Quality Improvement Project database for 2008–2014 was reviewed for patients who had undergone surgery for endometrial cancer. During the time period, the proportion of superficial SSI reduced from 4.5% to 1.9%, and deep incisional SSI reduced from 0.6% to 0.5%. Simultaneously, a significant implementation of minimally invasive surgery (24.2–71.4% of procedures) and a concomitant decrease in the use of open surgery (74.3–26.4% of procedures) was observed (Casarin et al., 2018). In the Bakkum-Gamez et al. study (Bakkum-Gamez et al., 2013), laparotomy comprised 86.9% of the operations, as opposed to 26% in our study.

Of the parameters associated with an increased SSI risk, only lymphadenectomy can be considered specific to hysterectomies with cancer as the indication for surgery, when in fact, smoking was the most apparent modifiable patient-related risk factor. Consequently, smoking cessation should be an important part of any SSI risk-reduction strategy in preoperative patient counseling.

The study is limited by possible patient selection and incomplete data acquisition bias owing to its retrospective nature. One conceivably meaningful variable not available for the analysis was the rate of hypothermia (Kurz, Sessler & Lenhardt, 1996). However, the variability of body temperature can be expected to be of a minor effect since maintenance of perioperative normothermia is a measure of quality performance at our institution. As a single-center investigation, the power of our study may be limited in detecting the effect of rare risk factors on SSI.

**6.4 STRENGTHS AND LIMITATIONS**

The main strength of studies I–IV is the large sample size and prospectively maintained comprehensive clinicopathologic database, including a long follow-up time. One of the end-points was disease-specific survival, which is the ideal
outcome of interest after a cancer diagnosis. Detailed clinicopathologic data allowed us to control for the most common confounding factors. Presumably, this mitigated the shortcomings associated with the retrospective design of the study.

During the patient collection period, as of January 2012, our institution changed strategy from routine pelvic and selective para-aortic lymphadenectomy to selective lymphadenectomy. This has resulted in a rather high total lymphadenectomy rate, 64.8%, including a relatively large proportion of patients with low-risk EC having undergone lymphadenectomy, facilitating the recognition of patients whose disease stages were higher than expected.

The limitations of all the studies (I–IV) are inherent to all retrospective single-center studies. It is infeasible to rule out the presence of confounding factors or selection bias in the study cohort. Nevertheless, the demographics and tumor distribution by histology and stage in our cohort mirror the cohorts from other industrialized countries (Crosbie et al., 2012, Lewin, Wright, 2011, Werner et al., 2012).

6.5 FUTURE PROSPECTS

The developed risk-scoring model will hopefully reduce LND rate in low-risk patients providing shorter operative times, fewer complications such as lymphedemas and SSIs and lower hospital costs. The relapse findings may encourage future studies in order to lessen relapses in early-stage high-risk EEC.

The ongoing debate about the role of lymphadenectomy will perhaps change when molecularly guided imaging or new biologic therapy becomes accessible to identify and treat systemic metastatic disease. Circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) represent an opportunity for the stratification of patients, for the assessment of early recurrent disease or for the real-time monitoring of therapy responses. Appropriately designed studies and implementation in clinical trials will determine the value of liquid biopsy for precision oncology in endometrial cancer. Molecular risk assessment has the potential to guide adjuvant therapy in integration with clinicopathological risk-stratification.
The present study on risk-profiling EC sought to develop a new operative risk-stratification model and evaluate risk factors for SSI after EC, resulting in the following conclusions:

I The developed risk-scoring system predicts stage IIIC–IV ECs with an NPV of 100%, which is highly sensitive in predicting an advanced-stage endometrial carcinoma at a cut-off of risk score points associated with an acceptable lymphadenectomy rate.

II The three compared risk models have similar accuracies in predicting lymphatic dissemination in endometrial cancer. The lymphadenectomy rate is lowest for the Milwaukee model. Risk variables included in the models predict patient outcomes independent of tumor stage.

III A relapse was diagnosed in 10.5% of patients, while the proportion of distant relapses was rather high at 57.1%. Uterine risk factors and positive peritoneal cytology are predictors of distant relapses. Patients with stage I cancer not satisfying low-risk criteria according to any of the models have poorer disease-specific survival than those with stage I low-risk cancer.

IV Organ/space infections comprise the majority of surgical site infections. Minimally invasive hysterectomy is associated with a smaller risk of incisional infections but not of organ/space infections.
This study was carried out in 2014–2018 at the Department of Obstetrics and Gynecology in Helsinki University Hospital. I wish to express my gratitude to the former and current academic Heads of Department of Obstetrics and Gynecology in Helsinki University Hospital, Professor Jorma Paavonen, and Professor Juha Tapanainen, and the former and current administrative Heads of Department, Docent Jari Sjöberg and Professor Seppo Heinonen, for their positive attitude toward research and for providing the academically motivating environment to work in.

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Helsinki, April 2019

Taru Tuomi
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