Novel preoperative biomarkers and evaluation of altered treatment strategies to improve outcome for endometrial cancer patients

David Forsse

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2021

UNIVERSITY OF BERGEN

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Date of defense: 01.10.2021

Year: 2021

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Print: Skipnes Kommunikasjon / University of Bergen

Scientific environment

The Bergen Gynecologic Cancer Research Group is a part of the Department of Clinical Science, University of Bergen. Offices and lab facilities are located in the Department of Obstetrics and Gynecology at Haukeland University Hospital. The group is led by Professor Camilla Krakstad and includes PhD students, postdoc fellows, research fellows as well as lab and study personnel. Professor Jone Trovik is PI for the ongoing Molecular Markers in the Treatment of Endometrial Cancer 2 (MoMaTEC2) international clinical study that emanates from this group.

The group maintains a comprehensive biobank with samples from gynecological cancer patients to be used in research, ongoing endometrial cancer organoid lines and animal model facilities to enable top-level translational research. Nearness to the clinic, including outpatient facilities and surgical theatre facilitates collection of biologic material and lays the foundation for new research ideas and collaborations with Helse Bergen in the cross-section of pre-clinical and clinical sciences.

The research group is a part of the Centre for Cancer Biomarkers (CCBIO), a Norwegian Center of Excellence, led by Professor Lars A. Akslen, which hosts state of the art research facilities and organizes activities and collaborations. The overall aim of CCBIO is to develop biomarkers to promote individualized cancer treatment.

The Bergen Gynecologic Cancer Research Group has close ties to the Mohn Medical Imaging and Visualization center (MMIV) and Bergen Abdominal Imaging research group led by Professor Ingfrid S. Haldorsen, which specializes in development and evaluation of radiological biomarkers for gynecological cancers.

Apart from MoMaTEC 2, a clinical multicenter study which involves centers from Norway, the Netherlands and Poland, there is ongoing participation in the European Network for Individualized Treatment in Endometrial Cancer (ENITEC) group, resulting in numerous collaborations. Other international partners include the Broad institute (Boston, USA) and the MD Anderson Cancer Centre.

The research group, the department and the university provide an unlimited source of inspiration and enables cancer research at all levels.

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With funding from

The Research Council of Norway

Momatec
Bergen Gyn Cancer Research

Centre for Cancer Biomarkers
Norwegian Centre of Excellence – University of Bergen

Acknowledgements

First of all, I would like to thank my supervisors, professors Camilla Krakstad and Jone Trovik, whose help has been invaluable when navigating through these years in pursuit of a PhD. Professor Krakstad offered me the chance to take a deep dive into gynecological oncology research and encouraged me to take a more clinical approach than initially foreseen. Thank you for helping me learn to navigate the academic labyrinth and learn how to perform in a scientific environment (and write shorter sentences)! Professor Trovik has been a great resource during the projects and writing processes that have led up to this thesis and has taught me that doctors need not fear statistics! Thank you for providing motivation and inspiration to combine academic and clinical careers.

I would like to thank my co-authors for their important contributions (in alphabetic order): Bjørg Almås, Stine Andreasen, Mark Barbero, Hege F. Berg, Elisabeth Berge Nilsen, Bengt-Erik Bertelsen, Olivera Bozickovic, Marie Ellstrøm Engh, Hilde Engerud, Kristine E. Fasmer, Ingfrid Haldorsen, Mari K. Halle, Erling Hoivik, Astrid Rege, Margaret Sævik-Lode, Ingvild L. Tangen, Ingvild Vistad, Kristin Viste, Kathrine Woie,

I have sincerely appreciated working together with my colleagues in the Bergen Gynecologic Cancer Research Group and office mates. During my three years of PhD studies some of you have come and gone. Ann-Helen, Camilla, Elin, Elisabeth, Erlend, Erling, Havjin, Hege, Heidi, Hilde, Hilde, Hildegunn, Ingfrid, Jenny, Jone, Julie, Kadri, Kari, Kim, Kristine, Mari, Marte, Marta, Madeleine, Ollie and Tina. You have all contributed to making our group a great place to work both professionally and socially, going to work has always been a pleasure!

Although representing a different field, a sincere thanks is in order to Alex for being my PhD-brother in arms, easily shifting from intense discussions on the meaning of interaction effects in linear mixed models to more testo-promoting and quality-of-life increasing activities, equally important!

A special thanks to Elisabeth Enge, who has put passion and effort into planning and effecting the follow-up of our gynecologic patients, always keeping the best of these women in mind and constituting the backbone in our effort to increase the knowledge on how treatment affects long-term quality of life.

A warm bunch of love to my family, my children Emil, Alva and Julia for making every day perfect, my brother Axel who secured his degree before me, making it impossible not to follow-up, and my parents Lisa and Erik for planting the seed of curiousness of science in me. Lastly, all my love to my life partner and best friend, Kristine.

Bergen, 25 june 2021

Abbreviations

AMPK	5' - adenosine monophosphate-activated protein kinase
ANOVA	Analysis of variance
AR	Androgen receptor
ARID1A	AT-rich interaction domain 1A
BSO	Bilateral salpingo-oophorectomy
CA-125	Cancer antigen 125
CI	Cervical stroma invasion
CT	Computed tomography
CTNNB1	Catenin beta 1
D&C	Dilatation and curettage
DJ-1	Parkinson disease protein 7, protein deglycase
DNA	Deoxyribonucleic acid
DSS	Disease-specific survival
EDTA	Ethylenediaminetetraacetic acid
EORTC	European organization for research and treatment in cancer
ER	Estrogen receptor
ERBB2	Erb-B2 receptor tyrosine kinase 2
FACT-G	Functional assessment of cancer therapy - General
FDA	Food and drug administration
FDG	Fluorodeoxyglucose
FIGO	International federation of gynecology and obstetrics
GDF-15	Growth/differentiation factor 15
GR	Glucocorticoid receptor
GSEA	Gene set expression analysis
HE4	Human epididymis protein 4
HER2/Neu	Human epidermal growth factor receptor
IARC	International agency for research on cancer
IGF-1	Insulin-like growth factor-1
IGF1R	Insulin-like growth factor 1 receptor
IR	Insulin receptor
JGOG	Japanese gynecologic oncology group
KRAS	K-Ras proto-oncogene, GTPase
LC-MS/MS	Liquid chromatography -tandem mass spectrometry
MI	Myometrial invasion
MMR-D/P	Mismatch repair deficient/proficient
MoMaTEC2	Molecular markers in the treatment of endometrial cancer 2 (study)
MRI	Magnetic resonance imaging
MSI-H	Microsatellite instability high
mTOR	Mammalian target of rapamycin
OS	Overall survival
PARP	Poly (ADP-ribose) polymerase
PD-1	Programmed cell death protein 1
PET	Positron emission tomography
PI3K	Phosphoinositide-3-kinase

POLE	Polymerase ε
PORTEC	Postoperative radiation therapy for endometrial carcinoma (study)
PPP2R1A	Serine/threonine-protein phosphatase 2A regulatory subunit A
PR	Progesterone receptor
PRO	Patient-reported outcome
PTEN	Phosphatase and tensin homolog
QLQ	Quality of life questionnaire
RAINBO	Refining adjuvant treatment in endometrial cancer based on molecular profile (study)
RCT	Randomized clinical trial
RFS	Recurrence-free survival
RNA	Ribonucleic acid
SAM	Significance analysis of microarrays
SEPAL	Survival effect of para-aortic lymphadenectomy in endometrial cancer (study)
SISAQOL	Setting international standards in analyzing patient-reported outcomes and quality of life endpoints (group)
TCGA	The cancer genome atlas program
TMA	Tissue microarray
TP53	Encodes p53 (tumor suppressor)
VEGF	Vascular endothelial growth factor
WHO	World health organization

Abstract

Background: Endometrial cancer is the most common gynecological cancer among women in countries with a high developmental index, and the incidence is expected to rise. Major controversies in the treatment of endometrial cancer revolve around the identification of women at risk of recurrence and optimal modes of treatment to minimize this risk. In addition, optimizing treatment-related quality of life is gaining attention. In recent years, several biomarkers have been identified and gradually implemented through changes in treatment algorithms, but further refinement is needed. Also, continuous evaluation of the resulting treatment changes is vital to improve survival and quality of life for endometrial cancer patients.

Aims: The overall aim was to improve endometrial cancer treatment through better preoperative stratification and evaluation of the effects of different treatment modalities on survival and morbidity.

Methods: In **Paper I**, 100 postmenopausal patients were selected from a population-based cohort, reflecting the clinical characteristics of the whole cohort. Preoperative blood samples were analyzed by liquid chromatography-tandem mass spectrometry, using a clinically implemented steroid hormone panel. Steroid levels were related to survival, tumor characteristics, radiologic assessment of fat distribution and gene expression**.**

In **Paper II,** all consenting endometrial cancer patients receiving primary treatment at Haukeland University Hospital over the period 2001-2019 were reviewed with a focus on comparing outcomes before and after implementing major treatment changes. These treatment changes were 1) a discontinuation of radiotherapy as an adjuvant treatment from 2009 (due to changes in national guidelines) and 2) a local initiative to implement a biomarker- and imaging-based selective lymphadenectomy policy in 2012-2013 to reduce the rate of patients undergoing lymphadenectomy. We assessed recurrence and survival and performed a trend analysis of changes in clinical and pathological factors over the time period.

In **Paper III**, we determined the effects of treatment modalities on quality of life and treatment-related symptoms in Norwegian patients enrolled in the ongoing Molecular Markers in the Treatment of Endometrial Cancer 2 (MoMaTEC2). Patients were grouped by received treatment modalities. Patient-reported outcomes at baseline and one and two years postoperatively were analyzed and compared to a Norwegian reference population. We used linear mixed models to assess the individual contribution of different treatment modalities.

Results: Low preoperative levels of 17-hydroxyprogesterone, 11-deoxycortisol and androstenedione were associated with aggressive tumor characteristics and poor disease-specific survival. 17-hydroxyprogesterone and 11-deoxycortisol were associated with prognosis independently of preoperative histological type and grade. Gene expression analysis revealed that tumors in patients with lower levels of these hormones expressed gene sets related to proliferation and cell cycle progression to a higher degree, whereas tumors in patients with higher levels expressed more inflammation-related genes. Higher levels of estrone and estradiol were associated with higher levels of body fat, expression of hormonal receptors and estrogen signaling-related gene expression, but not with survival **(Paper I)**.

After omitting radiotherapy as an adjuvant modality, 5-year overall survival increased in FIGO stage III (0.49 to 0.61, p=0.04) and recurrence-free survival increased from 0.51 to 0.71 (p=0.03). In other stages, survival outcome was maintained. For patients with stage I high-risk disease, the rate receiving adjuvant chemotherapy increased from 40% to 79%, but was not associated with any gain in survival **(Paper II)**.

The proportion of patients undergoing lymphadenectomy was reduced from 78% in 2001-2012 to 53% in 2013-2019 ($p<0.001$), with a maintained proportion of all patients with lymph node metastasis (9% versus 8%, $p = 0.58$). Patients not undergoing lymphadenectomy after 2012 were signified by low-intermediate risk based on MRI and histology of preoperative samples, negative PET/CT imaging and ER/PR positivity. Stage I patients, not undergoing lymphadenectomy, had maintained recurrence-free survival when comparing the time periods **(Paper II)**.

We found quality of life and functioning in endometrial cancer survivors comparable to a healthy age- and sex-matched cohort but significantly lower at baseline and increasing at year one and two post-operatively. Patients treated with adjuvant chemotherapy reported more tingling/numbness, lymphedema, and muscular pain at follow-up. There were no observable differences between patients in the groups not receiving chemotherapy (with or without lymph node staging). In multivariable mixed models, adjuvant chemotherapy was associated with tingling/numbness, lymphedema, fatigue and reduced physical functioning (**Paper III**).

Conclusions: Blood steroids have prognostic value, can be assessed from a preoperative blood sample with existing routine methods and may provide additive value to established preoperative biomarkers **(Paper I)**.

Replacing adjuvant radiotherapy with adjuvant chemotherapy had no negative impact on survival and showed improved survival for stage III patients **(Paper II)**. However, a marked increase in chemotherapy to stage I high-risk patients was not accompanied by an improved survival or recurrence rate, indicating an important area for further stratification of patients by biomarkers **(Paper II).** A selective lymphadenectomy algorithm based on hormonal and imaging biomarkers allowed for a substantial reduction of patients undergoing lymphadenectomy. The rate of patients with diagnosed lymph node metastasis and recurrence-free survival was maintained **(Paper II)**.

Overall quality of life is good for endometrial cancer patients. The group receiving adjuvant chemotherapy, however, reported increases in several symptoms, whereas patients undergoing lymphadenectomy without receiving chemotherapy did not. Removal of lymph nodes to select patients for adjuvant therapy therefore seems justified from the patient's viewpoint **(Paper III)**. In addition, the combination of unchanged survival and worse symptoms for early-stage patients receiving adjuvant chemotherapy warrants more focus on ways to optimize treatment for this group **(Paper II/III)**.

List of Publications

I. **Forsse D***, Tangen IL*, Fasmer KE, Halle MK, Viste K, Almås B, Bertelsen BE, Trovik J, Haldorsen IS, Krakstad C. Blood steroid levels predict survival in endometrial cancer and reflect tumor estrogen signaling. Gynecol Oncol. 2020 Feb;156(2):400-406. doi: 10.1016/j.ygyno.2019.11.123. Epub 2019 Dec 6. PMID: 31813586

II. **Forsse D**, Berg HF, Bozickovic O, Engerud H, Halle MK, Hoivik EA, Woie K, Werner HMJ, Haldorsen IS, Trovik J, Krakstad C. Maintained survival outcome after reducing lymphadenectomy rates and optimizing adjuvant treatment in endometrial cancer. Gynecol Oncol 160(2): 396-404. doi: 10.1016/j.ygyno.2020.12.002. Epub ahead of print. PMID: 33317908.

III. **Forsse D**, Barbero ML, Werner HMJ, Woie K, Nordskar N, Berge Nilsen E, Ellstrøm Engh M, Vistad I, Rege A, Sævik-Lode M, Andreasen S, Haldorsen IS, Trovik J, Krakstad C. Longitudinal effects of adjuvant chemotherapy and lymph node staging on health-related quality of life and patient-reported outcomes in endometrial cancer survivors. Submitted Manuscript to American Journal of Obstetrics and Gynecology.

*: These authors contributed equally.

Contents

1. Introduction

1.1 Epidemiology of endometrial cancer

1.1.1 Incidence

Endometrial cancer, arising in the epithelial lining of the uterus, is the most common of the gynecological cancers in countries with high developmental index, and is the 4th most common cancer among women in Europe and Northern America[1]. In Norway, approximately 750 new cases are diagnosed annually, resulting in a lifetime incidence around 2%, similar to other countries with high developmental index[2] (Figure 1). Over the last decades, many countries have reported increased rates of endometrial cancer, also when adjusting for increasing age and rates of hysterectomy[3, 4], and a further increase is expected due to increasing obesity[5]. Endometrial cancer is mainly a disease of postmenopausal women, with a median age at diagnosis of 68 years in Norway[2]. A substantial portion of the population has comorbidity and disability that needs to be considered when planning treatment[6].

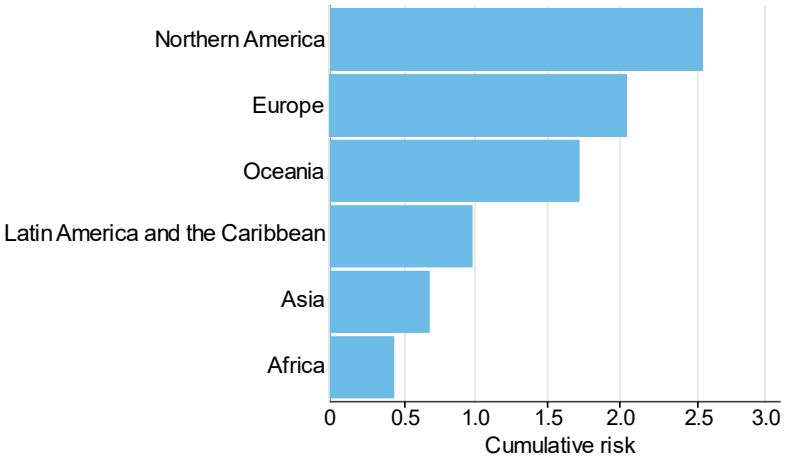

Figure 1. Estimated cumulative risk of endometrial cancer in 2020, up to age 74 in different continents. Source: IARC, Globocan 2020, https://gco.iarc.fr/ (with permission)

1.1.2 Risk factors

The Bohkman classification of endometrial cancer from 1983 describes two main types, and is still important for understanding the principal clinical division of endometrial cancer[7]. Type I, representing 80% of tumors, is estrogen dependent, has a lower median age of diagnosis and carries a better prognosis, whereas Type II tumors are more aggressive and are generally less dependent on estrogen exposure. The Bohkman classification has been replaced by more precise histological morphology in research and clinically, with endometrioid endometrial cancer roughly representing type I and non-endometrioid endometrial cancer representing type II tumors. The distinction can be unclear in endometrioid tumors with low differentiation and some non-endometrioid subtypes, but ongoing research into molecular subtypes is gradually providing a better understanding of connections between risk factors, histological morphology, and clinical characteristics. Still, influence of the female reproductive hormones is the most important mechanism through which risk factors of endometrial cancers can be understood (Table 1). Most epidemiological research on endometrial cancer risk factors has not discriminated between histological types, and there is reason to assume that endometrioid tumors are better represented in these statistics than non-endometrioid, as they are more common. There is evidence of some hormonal influence also on non-endometrioid tumors, albeit not to the same extent as for endometrioid endometrial cancer[8].

1.1.2.1 Unopposed estrogen

Healthy endometrium is an active tissue that responds to endocrine signals to accommodate reproduction during the fertile years. Estrogens and gestagens are endogenous sex hormones produced by the ovaries to control the cyclic endometrial transformation, with estrogen acting as a mitogen, inducing endometrial proliferation, whereas progesterone induces differentiation and maturation[9]. Withdrawal of progesterone after a period of exposure leads to shedding of the endometrium to prepare for a new reproductive cycle. This provides a natural protective mechanism against endometrial cells thriving long enough to accumulate oncogenic mutations. It has long been known that estrogenic exposure without balancing progesterone increases the risk of hyperplasia with increasing cellular atypia and finally cancer[10].

Table 1. Clinical Risk Factors for endometrial cancer grouped by main (hypothetical) pathogenic mechanism.

Factors increasing risk	Factors decreasing risk
Genetic risk - Lynch syndrome[11], Cowden syndrome[12] - First-degree relative with endometrial cancer[13] Endogenous hyperestrogenic balance - Obesity[14, 15] - Years of menstruation[16] - Nulliparity[17-19] - High concentrations of estrogens post-menopause[20, 21] Exogenous estrogen - Long-term use of tamoxifen[22] - Hormone-replacement therapy with less than 12–14 days of gestagens[23]	Decreasing estrogen/promoting gestagen: - Grand multiparity[17-19] - Smoking[24] - Oral-contraceptive use[25, 26] - Older age at last birth[27] - Breastfeeding[28] - Physical activity[29] - Diet of some phyto-estrogens[30]

Higher levels of endogenous circulating estrogens and their precursors increase the risk of endometrial cancer[20, 21, 31-33]. Exposure to exogenous estrogen or related compounds, (e.g. Tamoxifen) further increases the risk, while gestagen supplement can protect from or even resolve early cancer[34-36].

1.1.2.2 Obesity and endometrial cancer

Endometrial cancer risk increases with around 60% per 5 unit increase in body mass index, unparalleled by any other cancer type[15]. The strong link between obesity and endometrial cancer is multifaceted (Figure 2). Human adipocytes contain aromatase which can metabolize circulating androgen to estrogen leading to inhibition of normal endocrine cyclicity and anovulation, the unopposed estrogen mechanism. In addition, endogenous steroid levels could be further boosted by lack of sex-hormone binding globulins in obese individuals, and increased action of insulin-like growth factor and insulin resistance increase risk of endometrial cancer independently of estrogen[37, 38]. The relationship between obesity and endometrial cancer is likely even more complex

with adipokine-mediated influence and adipose-tissue mesenchymal stem cells that can be recruited to support the tumor[39, 40].

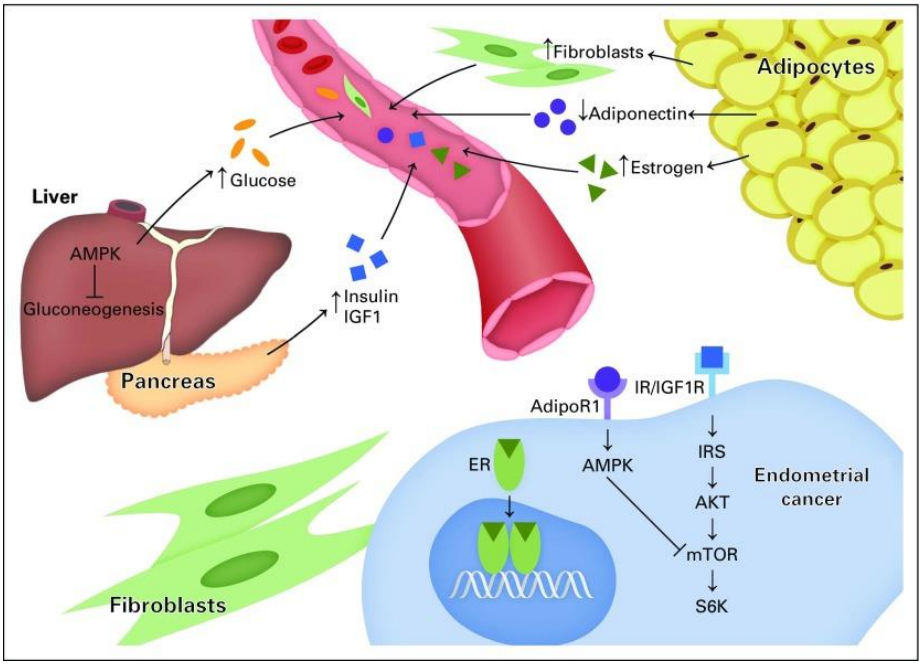

Figure 2. The oncogenic mechanisms of obesity in endometrial cancer. Adipocytes provide increased estrogen levels through androgen aromatization and alter the inflammatory environment through release of cytokines. Increased levels of estrogen, glucose, insulin, and insulin-like growth factor-1(IGF1) stimulate tumor growth through activation of mitogenic pathways. Furthermore, mesenchymal fibroblasts with stem cell properties can be recruited from adipose tissue to provide support in the tumor microenvironment. AMPK,5'-adenosine monophosphate-activated protein kinase; ER, estrogen receptor; IGF1R, insulin-like growth factor 1 receptor; IR, insulin receptor; IRS, insulin receptor substrate; mTOR, mammalian target of rapamycin. Illustration created by Suety Kwan, reprinted from Onstad et al. (2016) with permission[39].

1.1.2.3 Hereditary risk factors

Lynch syndrome, or Hereditary Non-Polyposis Colorectal Cancer syndrome, results from germline inactivating mutations in genes coding for specific DNA repair proteins. The function of these mismatch-repair (MMR) proteins (MLH1, MSH2, MSH6 and PMS2) is to resolve errors that arise in DNA replication, and deficiency results in a high number of mutations arising in a specific pattern; microsatellite instability

(MSI)[41]. Lynch syndrome is one of the most common inheritable causes of cancer, affecting cancer risk in diverse organs[42, 43]. In women with Lynch syndrome, endometrial cancer is the most prevalent initial site of manifestation, not rarely presenting at an early age. It is estimated that around 3% of endometrial cancer in unselected populations is attributable to Lynch syndrome, with higher prevalence in younger women[11, 44]. Diagnosing Lynch syndrome allows for proper surveillance and likely improves survival[45]. Prophylactic surgery has been shown to reduce endometrial cancer risk and is cost-effective[46, 47].

Hereditary inactivating mutations of the Phosphatase and tensin homolog (PTEN) suppressor gene are rare and give rise to the PTEN hamartous tumor syndrome (including Cowden syndrome). Patients with this disorder have an increased risk of several cancer types, among these a risk of endometrial cancer at 21-28%[12].

1.1.3 Survival

Long-term survival is excellent in early-stage endometrial cancer as the disease can be surgically removed by hysterectomy in about 85% of patients, yielding 5-year relative survival rates at 97 % for localized disease, and 87% for all patients[2] (Norwegian data, adjusted for expected mortality from other causes). Despite good prognosis, in some early-stage patients, the disease will recur, and make up a significant proportion of patients requiring non-surgical treatment. For patients with locally advanced or metastasized disease, prognosis is more dismal with 5-year relative survival rates of 68 % and 44 %, respectively (Figure 3).

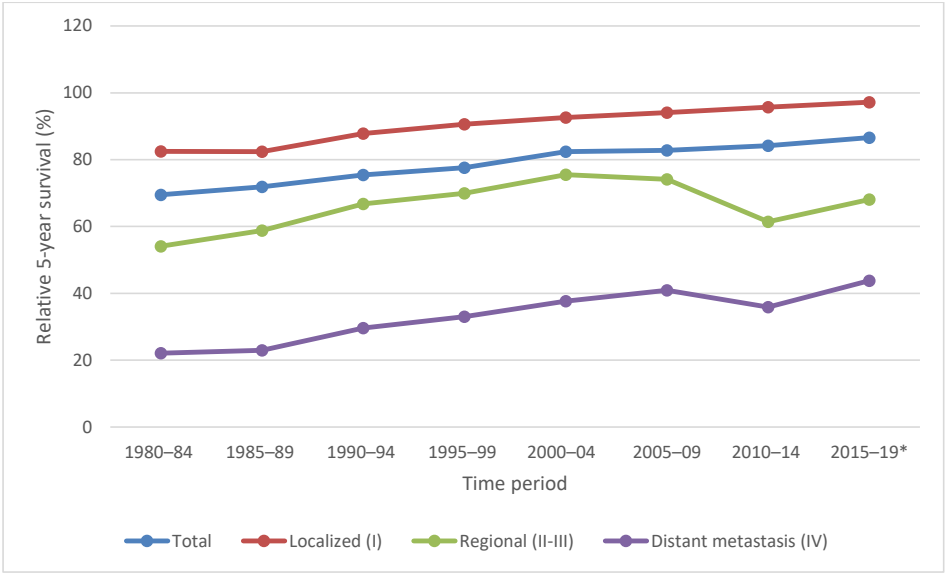

Figure 3. Five-year relative survival rates for Norwegian endometrial cancer patients, adjusted for expected mortality. Grouping is based on Surveillance Epidemiology and End Results Program (SEER) stage. Approximate corresponding International Federation of Gynecology and Obstetrics (FIGO) stage in parathesis. Source: Cancer in Norway 2019[2].

1.2 Symptoms and diagnosis

1.2.1 Presenting symptoms

As endometrial cancer grows in the uterine cavity it may cause vaginal bleeding, and postmenopausal bleeding is estimated to be the presenting symptom in 90% of patients[48]. In women presenting with postmenopausal bleeding, approximately 10% will have endometrial cancer[49]. Thus, all postmenopausal women with vaginal bleeding should have an examination to rule out cancer, generally by vaginal ultrasound and a biopsy. Endometrial thickness, as assessed by vaginal ultrasound, has been used as a stratification to allocate patients with postmenopausal bleeding to endometrial sampling, but sampling should be performed liberally, at least in women > 60 years[50]. In pre- and perimenopausal women, bleeding irregularity can be a symptom of endometrial cancer. A minority of patients will present with symptoms from metastasis without vaginal bleeding, in those cases, bowel symptoms, abdominal distension and pelvic pain may be present. Finally, a portion of endometrial cancer patients are diagnosed without symptoms, either through follow-up of abnormal cervical screening tests, suspect imaging findings or after pathological examination of a presumed benign hysterectomy specimen. There are no routine screening programs for endometrial cancer, and studies have failed to show a better prognosis for patients diagnosed without symptoms than for those with bleeding[51, 52], implying that detection at debut of symptoms is adequate as a population strategy.

1.2.2 Diagnosis

The endometrial cancer diagnosis is based on a histological assessment of an endometrial tissue sample. Traditionally, the gold standard for endometrial assessment is a dilatation of the cervix and curettage of the entire endometrial lining (D&C), requiring anesthesia. During the last 20-30 years, devices for endometrial sampling in outpatient settings have been developed and gained popularity (pipelle, tao brush, etc.) with performance statistics comparable to D&C for the detection of endometrial cancer[53, 54]. The amount of tissue retrieved by sampling is generally small, and histopathological diagnosis can be limited or unclear. A full D&C can be performed in these cases to retrieve enough material for typing, grading and biomarker analysis and

should be performed in symptomatic patients with negative or inconclusive endometrial biopsies where there is clinical suspicion of cancer. A stenotic cervix can also mandate a dilatation under anesthesia to retrieve endometrial tissue. Hysteroscopy for the diagnosis of endometrial cancer has been studied and is shown to diagnose focal (pre)cancer in up to 6% of sampling-negative patients[55], but the simplicity and reliability of a clinical evaluation with ultrasound and endometrial biopsy makes it unnecessary in most situations. Although hysteroscopy may increase the dissemination of tumor cells to the peritoneum, this does not worsen prognosis[56, 57].

1.2.3 Pre-treatment risk assessment

1.2.3.1. Histological assessment

Currently, the main value of the endometrial biopsy is in diagnosing the disease and stratifying the tumor according to histological appearance (Figure 4). The World Health Organization (WHO) Classification of tumors is generally recommended for classification[58]. The histological type of the tumor derives from its morphology and is associated with prognosis. Endometrioid endometrial cancer is the most common type, constituting roughly 80% of cases. Endometrioid cancers are traditionally graded according to the three-tier FIGO grading system, where higher grade signifies less glandular differentiation (and/or more nuclear atypia) and poorer prognosis. A binary grading system, grouping grades 1-2 as low risk and grade 3 as high risk, is more clinically relevant as distinguishing between grade 1 and 2 endometrioid tumors rarely affect treatment planning[59]. Among non-endometrioid subtypes, serous endometrial cancer is the most frequent, followed by clear cell cancers and carcinosarcomas. The non-endometrioid histological types are all considered high risk and are associated with a higher rate of extrauterine spread at diagnosis, carry poorer prognosis and require more aggressive treatment. More rare histological types exist such as dedifferentiated, undifferentiated and mixed carcinomas, and are generally classified high risk. Low interobserver reproducibility in distinguishing serous and high-grade endometrioid tumors is an important issue in endometrial cancer pathology with disagreement present in around 30%[60-62], and further refinement is needed to approach the histological reproducibility attained in ovarian or breast cancer[63]. Another problem is the lack of correlation between preoperative and final histopathological diagnosis, with

agreement as low as 67%, likely due to limited sampling preoperatively and tumor heterogeneity[64]. Assessment of biomarkers in the preoperative sample will be discussed in the chapter on precision medicine.

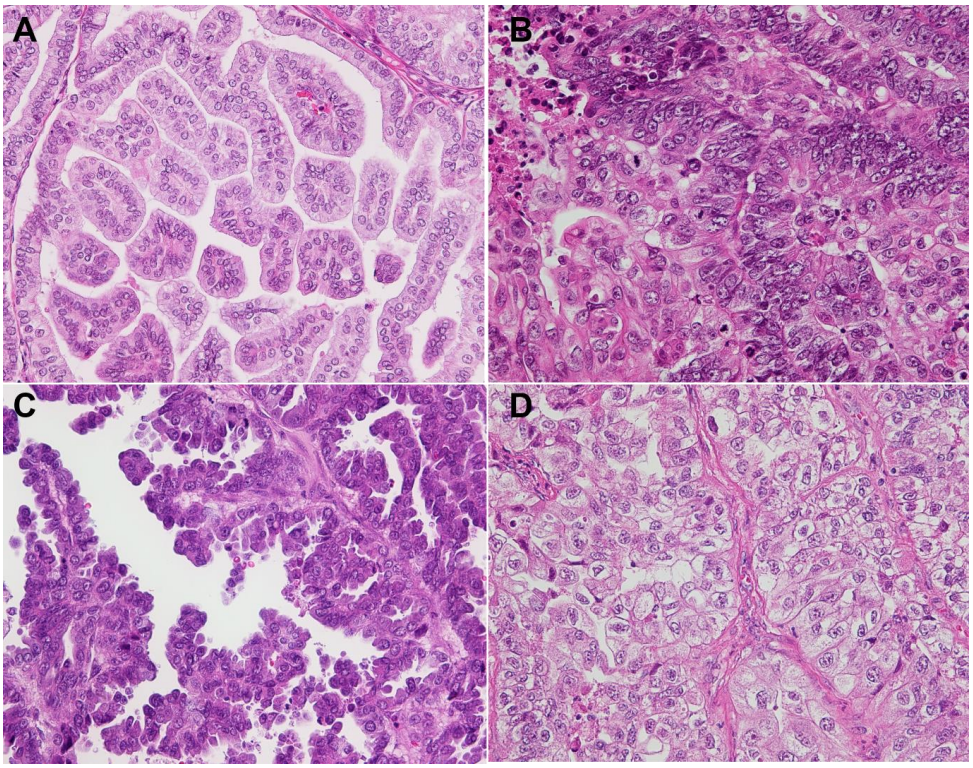

Figure 4. Histopathological subtypes of endometrial carcinoma. A) Endometrioid carcinoma grade 1. B) Endometrioid carcinoma grade 3. C) Serous carcinoma. D) Clear cell carcinoma. All images in 400x magnification, courtesy of Karen Mauland.

1.2.3.2 Preoperative imaging

Imaging modalities are used to assess the extent of endometrial cancer preoperatively to plan treatment or to assign stage to patients in whom surgery is not an option. Findings reported from preoperative imaging correspond to the surgico-pathological FIGO 2009 staging system, where important parameters are degree of myometrial invasion (MI), cervical stroma invasion (CI) and metastatic spread to adjacent organs, lymph nodes or distant organs[65].

Transvaginal ultrasound is integral in the gynecological exam used in the primary assessment of endometrial cancer patients. Apart from being used to diagnose the disease, it can be used to assess MI, CI and surgical mobility of the uterus. Magnetic resonance imaging (MRI) has emerged as a state-of -the-art imaging modality for pelvic tumors, as it avoids bony artefacts seen with computed tomography (CT) and provides high resolution in assessing MI and CI in uterine tumors[66]. Performance of transvaginal ultrasound to diagnose MI or CI, in the hands of expert operators (subspecialized gynecologists >6 years of experience), is comparable to MRI, but is poorer when handled by general gynecologists[67]. For diagnosis of extra-pelvic metastases, a preoperative CT is commonly used. 18F-FDG-Positron Emission Tomography combined with CT (PET/CT) has better sensitivity and specificity for detection of retroperitoneal lymphadenopathy and distant metastases, but is more expensive, and less available globally[68]. Ongoing research strives to identify novel radiologic biomarkers to improve prognostication and treatment for endometrial cancer patients. 18F-FDG uptake intensity is related to aggressive traits and may provide clinically useful information[69]. Other promising areas are artificial intelligence-derived radiological parameters and combinations with genetic tumor information; radiogenomics.

1.2.3.3 Blood samples

Clinical blood samples are obtained to assess the patient's health status preoperatively. Several blood biomarkers have been investigated in endometrial cancer, but none have reached wide acceptance as clinically useful. CA-125 is shown to have prognostic value and identifies advanced disease and lymph node metastasis to some degree, and HE4 is associated with an endometrial cancer diagnosis and higher stage[70-72]. Other blood-based biomarkers such as GDF-15 and DJ-1 have also been found promising[73, 74], but lack validation and clear clinical meaningfulness. Blood-based protein biomarkers may add value to multifactor models where they are combined with several other risk factors[75, 76]. Another area of intensive research is the detection of tumor material in blood, such as circulating tumor cells, tumor DNA or extracellular vesicles.

1.3 Treatment of endometrial cancer

1.3.1 Hysterectomy

In a majority of patients, the endometrial tumor is confined to the uterus and can be completely removed by surgically excising the uterus - a hysterectomy. Total hysterectomy, as opposed to amputating at the level of the cervix, is recommended for complete staging[66, 77, 78]. A radical hysterectomy, removing parametrial tissue and a 2 cm vaginal margin has not been shown to increase survival[79] and is not recommended in modern guidelines. A bilateral removal of salpinx and ovaries (BSO) is traditionally mandatory, but ovaries can be spared in selected premenopausal women without significantly affecting prognosis[80, 81]. In patients with advanced disease, when complete tumor removal is not attainable, debulking surgery is often performed, where removal of tumor tissue is performed to the limit of feasibility, including resection of abdominal organs, and affected peritoneum. In selected patients, a palliative hysterectomy can provide a solution to bleeding problems in the final stages of life.

1.3.2 Staging procedures

Surgical staging procedures, such as lymphadenectomy and omentectomy, do not on their own improve the prognosis for the patient. Instead, they serve to categorize patients into disease stages (Figure 5) according to the spread of the disease. In some cases, the results of staging will also affect adjuvant therapy, such as identifying lymph node metastases in a patient with presumed uterus-confined low-risk disease. Importantly, staging procedures increase operating time and risk of iatrogenic morbidity and should generally be restricted to where necessary.

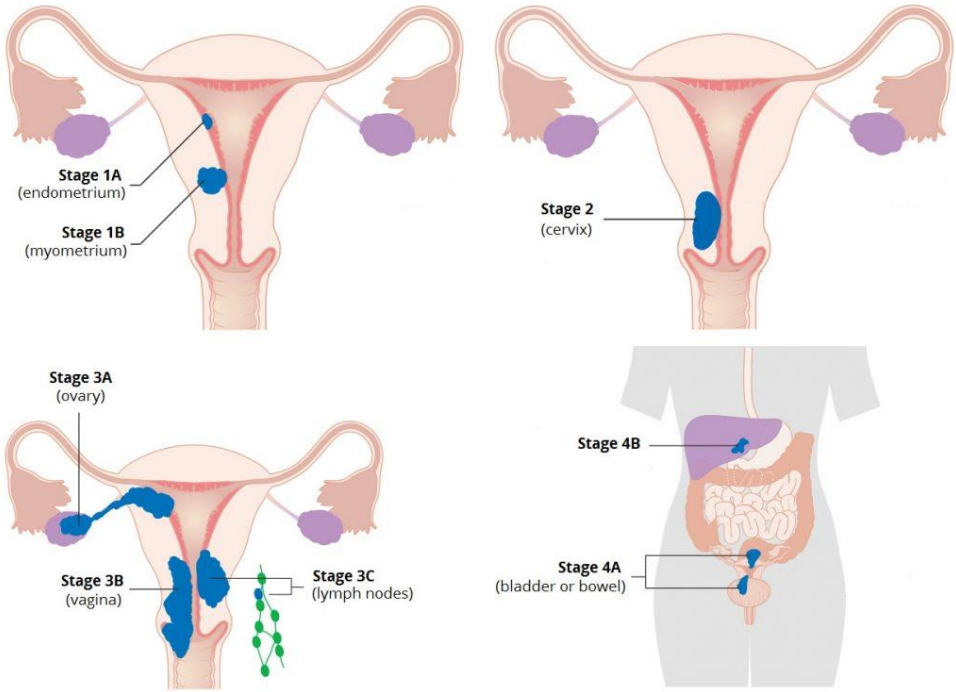

Figure 5. International Federation of Gynecology and Obstetrics (FIGO) Staging system for endometrial cancer. © Cancer Research UK [2002] All rights reserved. Information taken 11/06/21.

1.4.2.1 Lymphadenectomy

Lymphadenectomy is the removal of lymph nodes along the lymphatic pathways draining the uterus. In practice it is limited to the pelvic basin or extended to include para-aortic lymph nodes to the level of the inferior mesenteric artery or the renal vessels. The role of lymphadenectomy in endometrial cancer is controversial. Two large randomized clinical trials have concluded with no survival benefits of lymphadenectomy in endometrial cancer[82, 83]. However, important criticism has been raised, in part concerning low node counts for lymphadenectomies and unstandardized adjuvant regimes, that draw the conclusions into question. Interestingly, Naumann and colleagues performed a decision analysis suggesting that the studies were flawed by design and would not have been able to show benefits of lymphadenectomy even if these existed[84]. Other studies have documented survival benefits that correlate to the

number of lymph nodes removed[85, 86]. In the Survival effect of para-aortic lymphadenectomy in endometrial cancer (SEPAL) study, which was retrospective and with center bias, patients who went through pelvic and para-aortic lymphadenectomy had better survival than patients receiving only pelvic lymphadenectomy[87]. These findings are now being tested prospectively in the randomized JCOG1412- study (UMIN clinical trials registry id: UMIN000025399).

Lymphadenectomy increases the risk of perioperative complications such as blood loss, and postoperative lymphedema and lymphocyst formation, which can give long-term problems and affect quality of life[88, 89]. To weigh the importance of correct staging and tailoring of adjuvant treatment against the risk of inducing morbidity, preoperative algorithms have been developed to select patients at higher risk for lymphatic spread for lymphadenectomy while omitting it in those with lower risk.

1.4.2.2 Sentinel node biopsy

Sentinel node biopsy is rapidly gaining popularity in endometrial cancer as a replacement for lymphadenectomy for surgical staging[90]. Briefly, injection of a tracer in the uterine cervix allows for mapping of draining lymphatic pathways and the identification of the first encountered (sentinel) lymph nodes[91]. A sentinel node biopsy algorithm (including ipsilateral lymphadenectomy in case of failed mapping) is shown to have excellent performance in the detection of lymph node metastasis, with a sensitivity and negative predictive value reaching 98% and 99.8%[92]. Sentinel node biopsy does not affect oncological outcome compared to a comprehensive lymphadenectomy policy in retrospective studies[93]. Its strength lies in a reduction of peri- and post-operative complications[89], and it has been shown to be associated with lower cost and higher gain in quality of life adjusted years compared to systematic or selective lymphadenectomy in one study[94]. Although very promising, effective sentinel node biopsy relies on procedure experience and availability of equipment[91], and as of yet, no randomized trials comparing sentinel node biopsy to standard lymphadenectomy have reported results.

1.4.2.3 Omentectomy

Infracolic omentectomy is recommended for serous cancers and carcinosarcomas, as these are associated with a high rate of micrometastases to the omentum[95]. The risk of omental spread in presumed early-stage endometrioid endometrial cancer is very low and does not justify routine omentectomy[96]. There is some guideline divergence regarding the procedure for clear cell tumors[66, 77].

1.4.2.4 Other staging procedures

Peritoneal washings have traditionally been secured at the start of surgery to identify malignant cells outside the uterus. Positive washings led to an advanced stage diagnosis according to the FIGO system up until the 2009 revision, where it was removed, as data did not support an independent prognostic value[97]. Perioperative frozen section of the uterus, with evaluation of for example myometrial invasion, has been used to ascertain the need for further staging. It is deemed as obsolete by the latest European Society of Gynæcological Oncology guideline and is not mentioned as a staging technique in the National Comprehensive Cancer Network (USA) guidelines[66, 77].

1.3.3 Adjuvant treatment

Adjuvant treatment refers to non-surgical treatment given in addition to primary surgery to reduce the risk of relapse (or prolong progression-free interval). In endometrial cancer, the main modalities have been chemotherapy, radiotherapy and hormonal therapy. Because many patients are cured by surgery alone, and adjuvant therapies generally are associated with toxicity and reduced quality of life, there is consensus that adjuvant therapy should be restricted to groups of patients that likely benefit. Standard regimens are presented in table 2.

Adjuvant therapy policies have varied greatly between institutions, generally motivated by tradition and interpretation of available data. In Norway, adjuvant radiotherapy was generally discontinued after a randomized controlled trial (RCT) demonstrating better survival for adjuvant chemotherapy-treated patients than those receiving whole abdomen irradiation[98]. Two other RCTs have compared these modalities, finding no survival difference[99, 100]. Heterogeneity in the composition of chemotherapy and irradiation technology make comparisons challenging and interpretations uncertain.

Table 2. Standard adjuvant therapy regimens for endometrial cancer. Examples of regimens from PORTEC-2, PORTEC-3 and GOG-258[101-103]. In Norway, chemotherapy is the preferred adjuvant modality.

Modality	Standard content	Number of treatments	Duration
Chemotherapy	paclitaxel 175mg/m2 + carboplatinum AUC 5-6	6	18 weeks
External beam radiation therapy (EBRT)	1.8 Gy fractions directed to pelvic area, aortal field can be included, brachtherapy boost can be included	25-27	5-6 weeks
Brachytherapy	Dose delivering isotope inserted in vaginal vault for long (LDR) or short (HDR) duration	2-6	3 weeks
Chemoradiotherapy	EBRT + concomitant cisplatin 50mg/m2 x 2 + post-radiation paclitaxel 175mg/m2 + carboplatin AUC 5-6 x 4	25-27 + 4	18 weeks
Hormonal therapy	Gestagen or anti-estrogen until failure	-	-

AUC, Area under curve

There is however data to show that local recurrence rates are reduced by radiotherapy, also compared to chemotherapy[98, 101, 104, 105]. This effect can be achieved also by brachytherapy, thereby reducing the radiation load delivered to healthy tissue[103]. Institutions avoiding upfront adjuvant radiotherapy may still benefit from its effect on local recurrences by offering it when the recurrence arises (salvage therapy), and there are no definitive data to support either of these radiotherapeutic strategies above the other. Recently, The PORTEC group demonstrated the combination of radiotherapy and chemotherapy to be more effective than radiotherapy alone for high-risk patients[102]. A comparable study conducted by Matei and colleagues did not find any difference between the same radiochemo regimen versus chemotherapy alone[101]. Hormonal therapy is not regarded as a first-line adjuvant treatment[66].

The application of molecular subgroups is likely to affect adjuvant therapy guidelines. Stratification to improve identification of those patients that most benefit from the treatment, and prospective trials to explore this are in progress, such as the RAINBO

(Refining Adjuvant treatment IN endometrial cancer Based On molecular profile) umbrella program and PORTEC-4a.[106]

1.3.4 Advanced or recurrent endometrial cancer

In patients with metastatic spread of endometrial cancer, treatment can consist of tumor reducing surgery, chemotherapy or radiotherapy, or combinations thereof. A comprehensive debulking is recommended if deemed feasible, combined with chemotherapy, radiotherapy or both[66]. The 5-year recurrence-free survival in this group is similar for patients treated with adjuvant chemotherapy and radiochemotherapy, but slightly poorer for radiotherapy alone[101, 102]. Carboplatin is preferred to cisplatin due to milder adverse effects, and the combination with paclitaxel is shown to be non-inferior to a triplet with doxorubicine, cisplatin and paclitaxel[107]. In cases where local spread makes resection impossible, neoadjuvant chemotherapy followed by surgery or definitive radiotherapy are options[66, 77].

Local recurrences can be excised if feasible and/or targeted with radiotherapy. Systemic treatment options for recurrent disease are limited to single agent or combination chemotherapy in patients with good performance status, or hormonal therapy. For the combination of carboplatin and paclitaxel in the recurrence setting, overall survival and progression-free survival is 37 and 13 months, respectively[107]. The response rate to hormonal treatment in around 25%, with up to 35% in hormone receptor positive patients[108, 109]. For retreatment with chemotherapy (where adjuvant chemotherapy was given after primary surgery), a small retrospective series showed partial response in 50%, with no complete responses and progression-free survival and overall survival of 10 months and 27 months respectively[110].

As new mechanisms of tumor biology are unraveled, novel targets for treatment can be identified. Thus, there is hope for improving treatment and subsequently prognosis for endometrial cancer patients in the future. This is discussed in the following two chapters.

1.4 Endometrial cancer biology

1.4.1 Important genetic alterations in endometrial cancer

Increasing understanding of the mechanisms that drive the development of malignant tumors have identified crucial properties that cells must acquire to prosper as cancer (for general reviews on key features of tumor biology, see [111, 112]). Genetic alterations are required to obtain these properties, and may follow distinct patterns based on germline features, mutagen exposure, qualities of the original somatic cell, and its environment[113, 114]. The development of tools to assess mutations genome-wide, such as massive parallel sequencing, has led to the identification of multitudes of possible tumorigenic genomic alterations and research is ongoing to clarify how these may be exploited in the treatment of cancer[113].

In endometrial cancer, specific recurring mutations have the potential to affect treatment decisions, with many promising applications[115, 116]. Of the most notable are alterations in the phosphatase and tensin homolog (PTEN) suppressor gene (present in 60-90% of endometrioid tumors), or in Phosphoinositide -3-Kinase (PI3K) proteins, that induce an uninhibited PI3K-Akt-mTOR signaling[116]. PTEN-mutations are frequently seen in endometrial hyperplasia, suggesting a role in early development, albeit not sufficient for malignant transgression[117, 118]. Other targetable mutations include TP53, CTNNB1, ERBB2, FGFR2, ARID1A, and KRAS, where alterations can be found in different histologic subtypes to varying degree[119]. For example, mutations in CTNNB1 commonly occur in low-grade endometrioid tumors and signify adverse prognosis, but are uncommon in non-endometrioid subtypes. TP53 mutation on the other hand is highly recurrent in serous endometrial cancer, and ERBB2 amplifications are rarely seen in other subtypes than serous[119].

Next generation sequencing and bioinformatic analysis of 373 endometrial cancer samples by the Cancer Genome Atlas (TCGA) research network identified four molecular subgroups with distinct prognosis[120] (figure 6). The first group, constituting 7% of tumors in the TCGA-study, were characterized by mutations in the exonuclease

domain of polymerase ε (POLE). This results in a defect DNA-synthesis proof-reading mechanism, and an ultra-high mutational rate. These tumors have an excellent

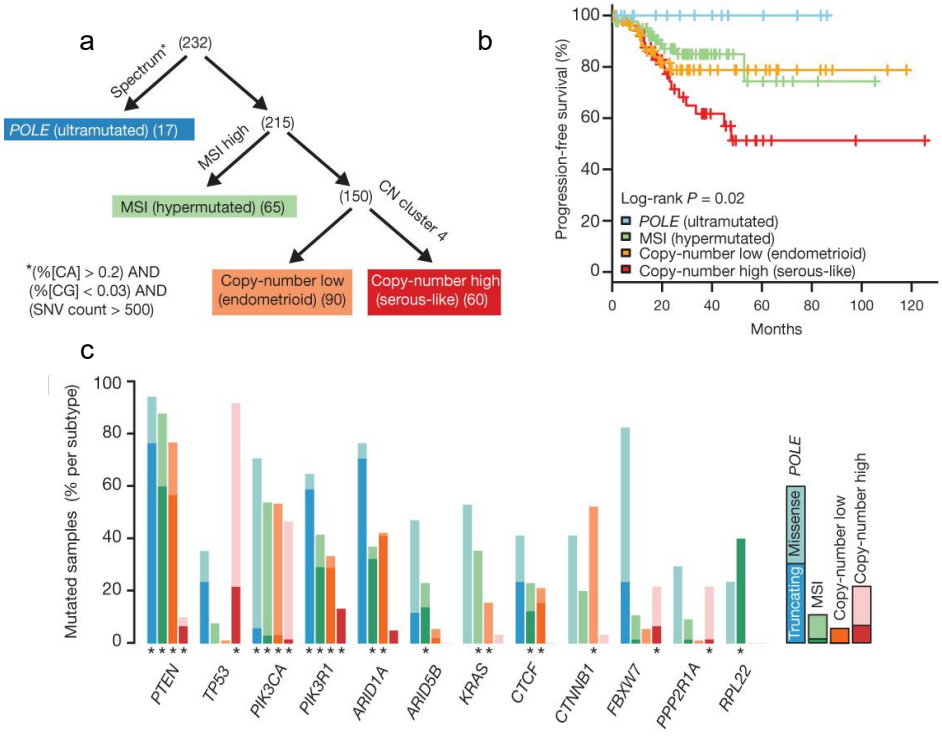

Figure 6. The Cancer Genome Atlas molecular subgrouping for endometrial cancer a) Tumors were stratified into four groups by nucleotide substitution frequencies and patterns, MSI status, and copy-number cluster. SNV, single nucleotide variant. b) POLE-mutant tumors have significantly better progression-free survival, whereas copy-number high tumors have the poorest outcome. c) Commonly mutated genes differ between the four subgroups. The mutation frequencies of all genes that were significantly mutated in at least one of the four subgroups are shown (asterisk denotes false discovery rate < 0.05). Adapted with permission from Levine at al 2013[120], under the CC-by-NC-SA 3.0 license.

prognosis, even in high grade endometrial cancer. Next, MSI-high (MSI-H) or MMR deficient (MMR-D) tumors have deactivating mutations in one of the MMR genes, resulting in a high mutation rate (but lower than POLE). Interestingly, this genetic alteration has important treatment consequences, as tumors may respond to immune

checkpoint inhibitors[121]. The remaining tumors were divided into two groups based on copy number alterations, with the copy number high group containing almost all serous tumors, in addition to some grade 3 endometrioid. This “serous-like” group is generally TP53-mutated and has the poorest prognosis of the groups. The final group, copy-number low, has an intermediate prognosis, akin to the MSI-H/MMR-D group, seems to contain a mix of the classical histological subtypes and lacks obvious identifying protein features. The TCGA classification is currently being adopted into clinical guidelines, with the aim to guide treatment[66, 77].

1.4.2 Estrogen signaling and hormonal receptors

The presence or absence of estrogen and progesterone receptors (ER/PR) are particularly important in endometrial cancer. In the normal active endometrium, estrogenic signaling drives the initial proliferative phase. Circulating estrogens produced by the leading ovarian follicle bind to cytoplasmic estrogen receptors that dimerize, enter the nucleus and act as transcription factors[122]. In addition, non-transcriptional effects are mediated via G-protein coupled membrane-bound receptors[123]. Increased expression of PR, also a member of the nuclear family of receptors, prepares the cells to relay progesterone signaling in the luteal phase. The expression of PR is induced by estrogen signaling. Progesterone inhibits the proliferative effects of estrogen and induces differentiation and maturation of tissues leading to decidualization of the endometrium[124]. Both ER and PR have subclasses with functional differences (ERα/ERβ and PR-A/PR-B), but the importance of these for endometrial cancer biology is not fully elucidated.

Epithelial expression of ER and PR is maintained in endometrial hyperplasia, the precursor of endometrial cancer, signifying maintained estrogen signaling. Generally, ER and PR are expressed in highly and moderately differentiated endometrioid subtypes (grade 1-2), whereas they are often lost in more aggressive tumors, such as grade 3 endometrioid, and non-endometrioid subtypes[125]. Although hormone receptors are absent, there may still be significant estrogenic activity[8].

1.4.3 The role of steroid hormones

The majority of endometrial cancer patients are post-menopausal with ceased ovarian hormone production. Nevertheless, residual endogenous estrogen levels vary (depending on phenotype) and can be affected by exogenous hormonal compounds. The metabolism of steroid hormones is complicated with many intermediate forms that may have effects on tumorigenesis[20, 31, 32] (Figure 7). Studies have shown that apart from highly active estrogenic compounds, other steroid hormones (such as the androgens testosterone and androstenedione) may increase the risk of endometrial cancer[21, 33, 38, 126]. Thus, the phenotypic steroid profile may contain more information on risk than that imparted by estrogen levels alone. Our group previously demonstrated differences in levels of several steroids in blood samples in a matched sample of long vs short surviving endometrial cancer patients[127]. This finding raises the question if circulating steroid hormones can be exploited for prognostic information or even predictive value in the treatment of endometrial cancer.

1.4.4 The hormonal microenvironment

Although a majority of endometrial cancers are hormone receptor positive and thought to be estrogen-driven through activation of tumor cell nuclear receptors, little attention has been given to the hormonal microenvironment surrounding the tumor. There is however data pointing to important hormone-stroma interaction effects that may further our understanding of the relationship between hormonal signaling and endometrial cancer and how to exploit this for therapy:

1. Stromal cells are directly involved in hormone signaling: A PTEN knockout endometrial cancer mouse model showed that loss of PR signaling in stromal fibroblasts was a mechanism of resistance to treatment with progestins. PR expression in the stroma could induce sensitivity to progestins in spite of epithelial (tumor) PR negativity[128].
2. Low stromal PR expression is associated with resistance to progestin treatment in complex atypical hyperplasia where epithelial PR expression is preserved[129]. Alteration of stromal hormone signaling may be an early component of tumorigenesis in hormone-driven endometrial cancer.

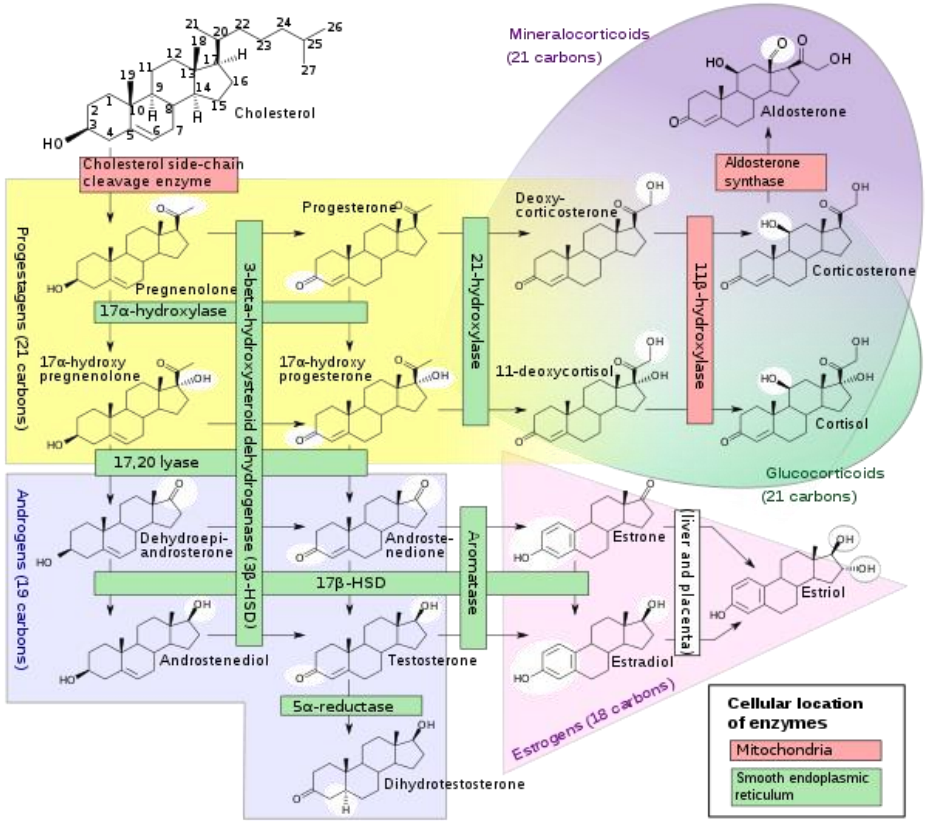

Figure 7. Metabolism of the major classes of steroid hormones with active enzymes. Background color signifies hormone action (partly overlapping). HSD, hydroxysteroid dehydrogenase. Reprinted with permission under CC BY-SA 3.0.[130]

3. Expression of hormone-altering enzymes is associated with aggressive tumor characteristics and prognosis in endometrial cancer[131]. Induction of stromal hormone-converting enzymes may be an important mechanism by which to increase mitogenic signaling[132].

Thus, circulating hormones may affect tumor cells indirectly and independently of epithelial receptor expression. There is hope that a deeper understanding of the stroma-hormone-tumor axis interactions may yield new insights to increase efficiency of existing hormonal treatment or lead to development of new therapeutic options in endometrial cancer.

1.5 Precision treatment in endometrial cancer

Precision treatment in cancer refers to tailoring treatment to properties of the patient or the disease[133]. This can be achieved either by identifying the patients that benefit from a given treatment or by designing treatment that targets specific molecules, signaling pathways, or functional alterations that arise in subgroups of a cancer.

1.5.1 Biomarkers for precision medicine

Identification of biomarkers is closely related to development of precision treatment. A cancer biomarker may be any measurable biologic entity that provides information on cancer parameters[134]. Biomarkers are generally classified according to their utility as either prognostic or predictive, meaning they are either useful for sorting patients according to survival or according to response to a predefined treatment. As prognostic biomarkers can be identified from observational studies without controlling allocation to treatment, these are more abundant in the literature[135].

1.5.2 Prediction of lymph node metastasis

Although endometrial cancer provides a unique possibility for retrieving tissue from the tumor prior to definitive treatment, few preoperative biomarkers have gained widespread use. A main reason for this is that most patients will undergo primary hysterectomy irrespective of risk assessment. Post-operatively, the complete primary tumor is available for analysis of histological risk factors which provides the gold standard. Possible applications for preoperative biomarkers are the selection of patients for non-surgical treatment or omitting staging procedures to minimize morbidity. In endometrial cancer research, identifying biomarkers to aid in the selection of patients to undergo lymphadenectomy has been a prioritized goal. For a biomarker to be effective in this setting, it needs to have a high sensitivity for lymph node metastasis and produce a low negative predictive value, minimizing the number of patients that are understaged, as this has important implications for adjuvant treatment and subsequently survival.

Although preoperative imaging has improved the ability to diagnose lymph node metastasis, it is limited by the size (and metabolic activity) of the metastasis[136].

Lymphovascular space invasion is a strong histological prognostic biomarker but is not assessable in preoperative biopsies[137]. L1CAM, ER, PR, and p53 are examples of easily assessed histological biomarkers that provide information on the risk of lymph node metastasis[125, 138, 139]. The ongoing Molecular Markers in the Treatment of Endometrial Cancer phase 4 multicenter study (MoMaTEC2) is investigating the effects of limiting lymphadenectomy to cases at increased risk of lymph node metastasis based on ER/PR-expression in a preoperative sample. It is plausible that combining multiple biomarkers in panels will improve their prognostic value, and that this may increase the clinical usefulness[75]. Molecular classification, for example as proposed by the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) initiative, has the potential to alter the preoperative assessment of endometrial cancer, and preoperative biopsy classification correlates well with the hysterectomy specimen[140, 141].

1.5.3 Targeted treatment in endometrial cancer

Immune checkpoint inhibitors are one of the true oncological precision medicine breakthroughs. These antibodies target interaction between cells of the immune system and the tumor and can reverse immune evasion properties in cancer cells. Pembrolizumab inhibits contact between PD-1 and its ligand in T-cell-tumor interaction, and has been approved in the USA for solid MSI-H tumors, endometrial cancer included[142]. These tumors exhibit mismatch repair deficiency (MMR-D)/microsatellite instability (MSI), resulting in a high mutational burden and high neoantigen load, which makes them susceptible to the T-cell immune defense. In addition, recently, the combination of levatinib (a VEGF inhibitor) with pembrolizumab (PD-1 inhibitor) has been approved by the FDA following the results of Keynote-146 for treating MMR-proficient endometrial cancer patients[143, 144].

Based on overexpression of HER2 in 30% of serous cancers, and their efficacy in breast cancer, the effect of HER2-targeting antibodies has been explored in endometrial cancer. A phase II study with carboplatin/paclitaxel with or without trastuzumab in advanced or recurrent serous carcinomas overexpressing Her2/Neu showed increased PFS and OS. Median overall survival was not yet reached in the trastuzumab arm after a median follow-up of 26 months[145]. It should be stressed that none of the above-

mentioned treatments have been validated at phase III level. No modern targeted therapies are in general use in Norway, but can be approved by a national "expert panel" evaluation system.

There is hope for novel approaches following the logic of the TCGA classification, apart from the link between MMR-D and Immune checkpoint inhibitors. POLE tumors have an ultra-high mutational load and are likely susceptible to immune checkpoint inhibition, but have inherently good prognosis, and less treatment is more likely to be the goal for this group[146]. For the copy number high (serous-like) subgroup, the TCGA study and pathology studies reveal a high grade of similarity to high grade serous ovarian cancer and basal-like breast cancer, also expressing homologous repair deficiency in many cases; this raises the question of a potential effect of Poly (ADP-ribose) polymerase (PARP) inhibitors for this subgroup. PARP inhibitors have shown in vitro effects, especially in sensitizing cells to chemotherapy, and several agents are being tested out in Phase I/II clinical trials[147]. The copy number low group contains a majority of endometrioid, ER/PR positive tumors, and while the molecular profile does not give obvious grounds for a specific targeted treatment, these tumors may be susceptible to hormonal treatment in the palliative setting. Also, this group had the highest occurrence of CTNNB1 mutations in the TCGA data (>40%), providing a promising target should an effective drug be discovered.

1.6 Quality of life

1.6.1 Living with cancer

After receiving primary treatment for endometrial cancer, most patients will be cured. Treatment-related morbidity and functional decline is highly important as it will affect quality of life for many years. Clinical follow-up is motivated by the possibility of discovering asymptomatic recurrences eligible for treatment, but the effect on survival is uncertain[148]. In contrast, follow-up is potentially an opportunity to assess post-treatment morbidity and improve quality of life for patients. To achieve this, data on what parameters to measure and how to interpret responses is needed. Follow-up schemes vary between healthcare systems and are usually tailored to the patient's wishes and available healthcare resources. In Norway, follow-up is normally planned for every 3-4 months during the first two years followed by every 6 months until 5 years post diagnosis[149].

For patients presenting with uncurable disease, the time-frame post treatment is shorter. Palliative treatment is intended to minimize discomfort during the final stages of the disease. Treatment should be carefully tailored to the patient's wishes and expectations in these situations. To achieve this, there must be available information on potential outcome related to different treatment modalities[150].

1.6.2 Treatment-related morbidity

As nearly all patients go through primary surgery including hysterectomy and BSO, reducing morbidity resulting from this procedure has been in focus. Surgical approaches, minimally invasive laparoscopy and traditional laparotomy, have been compared in large trials, and suggest better short-term quality of life for laparoscopy but with no obvious long-term differences[151, 152]. Lymphadenectomy is associated with lower-extremity lymphedema[88, 153-158]. Sentinel node biopsy seems a promising alternative, however, there is a lack of studies assessing differences in survival, quality of life, or morbidity when comparing sentinel node biopsy with selective lymphadenectomy. Estimates of lymphedema are hard to interpret due to lack of

standard criteria, influence of comorbidities, and possible dynamics in the course of the disease[159].

Longitudinal data on long-term effects of adjuvant radiotherapy is available and show persisting gastrointestinal problems up to 10-15 years[160-162]. For adjuvant chemotherapy, health-related quality of life outcomes have been used to ascertain advantage of using carboplatin plus paclitaxel over a cisplatin-doxorubicin-paclitaxel triplet[107], and comparisons have been made between chemotherapy, chemoradiotherapy and radiotherapy in treatment of advanced stage patients[101, 163]. Generally, these secondary endpoints address the short-term effects of the treatments and are used to find a preferred treatment when the survival outcome is equal. Less is known about long-term effects of adjuvant chemotherapy on endometrial cancer survivors.

1.6.3 Assessing morbidity and quality of life

In the assessment of health-related quality of life and morbidity, patient-reported outcome (PRO) is now regarded to constitute a highly valid endpoint, representing the patient's own assessment of the problem[164]. Alternative outcome parameters may be physician's opinion or objective measurements (e.g. leg circumference or pletysmography in the case of lymphedema), and although these have traditionally been seen as more objective, they are increasingly replaced by PRO assessments, transferring the power of definition to the patients. Specific assessments of for example lymphedema have been shown to correlate highly between patient-reported symptoms and objective measurement[165, 166]. The assessment of patient PROs such as perceived symptoms or functioning is useful in cancer research for several reasons:

- Exploring patient groups to address specific problems that are not acknowledged, e.g. sexuality in endometrial cancer patients.
- Developing methods for surveillance of patients with the aim to detect health issues that may be treated.
- Comparing treatment-related adverse effects in randomized trials, especially when survival gain is similar.

PRO assessment is generally based on questionnaires where the respondent is prompted to evaluate quality of life, functioning, and symptoms on Likert scales. These scores can then be summed or grouped to represent different entities of interest. Figure 8 shows an example of dimensions of a quality-of-life assessment from the European organization for research and treatment in cancer (EORTC)- quality of life questionnaires.

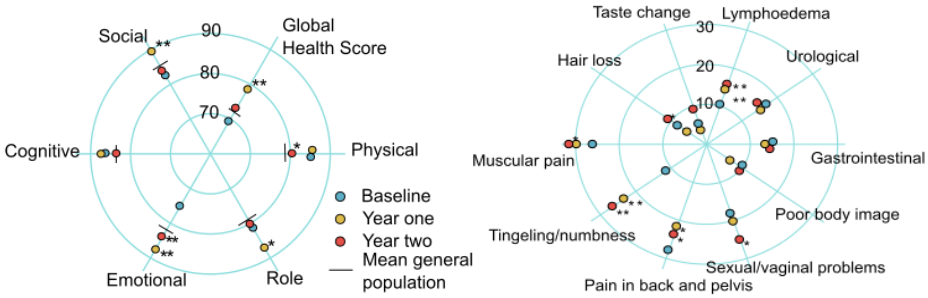

Figure 8. Radar plot of endometrial cancer patient means of EORTC-QLQ C30 and EN24 scales at baseline, year 1 and year 2. General population means are plotted as lines. Increasing score signifies better function. * p <0.05, **p<0.001. EORTC-QLQ, European Organisation for research and treatment of cancer-quality of life questionnaire. Adapted from Forsse et al. 2020 Poster at European organisation for medical oncology (ESMO) annual congress 2020. Unpublished results.

2. Aims of the study

2.1 Background

Endometrial cancer is a common female malignancy that requires prompt treatment for best prognosis. Most patients survive their diagnosis but may struggle with problems related to treatment. Aggressive disease requires more and tougher treatment, but precise methods to tailor treatment are unavailable. More targeted use of available treatment modalities that potentially affect morbidity is likely the most efficient way to improve survival and reduce morbidity for endometrial cancer patients. To improve endometrial cancer treatment, implementation of biomarkers in treatment algorithms as well as thorough investigation of treatment effects on survival and quality of life is vital.

2.2 Overall Aim

To improve endometrial cancer treatment through better preoperative stratification and evaluation of the effects of different treatment modalities on survival and morbidity.

2.3 Specific aims

Paper I: Determine the prognostic value of circulating steroids in endometrial cancer patients, and explore their additive value as a preoperative test to the current work-up.

Paper II: Assess the effects of 1) discontinuing adjuvant radiotherapy, and 2) reducing the rate of patients undergoing lymphadenectomy on recurrence and survival. In addition, we explored trends in clinical and pathological variables that could affect patient outcome during the observed period.

Paper III: Determine the effect of treatment modalities on differences in quality of life and patient-reported outcomes in patients subjected to hysterectomy alone, lymph node staging procedures and/or adjuvant chemotherapy.

3. Materials and methods

3.1 Patient series

3.1.1 The Haukeland cohort

Patients treated for endometrial cancer at Haukeland University Hospital have been prospectively included in a well annotated study cohort since 2001, primarily designed for identification of biomarkers in tumor tissue. The cohort is approved according to Norwegian legislation by the western regional committee for medical and health research ethics (REK 2014/1907, 2019/1020). Informed written consent has been obtained preoperatively from all included patients.

Haukeland University Hospital serves as a tertiary hospital for gynecologic oncology for the Vestland region, encompassing ~10% of the Norwegian patient population. The cohort is considered population-based as patient and disease characteristics reflect the nationally reported endometrial cancer statistics[2]. Tumor tissue samples, blood and urine are stored in the Bergen Biobank for Gynecological Cancer. A database with clinicopathological variables has been continuously updated based on patient file review and routine pathology reports. Prospective registration of recurrences and survival has been performed.

3.1.2 MoMaTEC2

Molecular Markers for Treatment of Endometrial Cancer 2 (ClinicalTrials.gov Identifier: NCT02543710) is an ongoing multicenter phase 4 implementation study, designed to evaluate the implementation of hormonal receptors as preoperative biomarkers to guide treatment. The study emanates from Haukeland University Hospital and includes several Norwegian hospitals (Figure 9). International participating sites are Nijmegen and Eindhoven (Netherlands) and Lublin (Poland) but included patients from these centers are not part of this thesis. Inclusion started in October 2015 and the study is still enrolling, aiming to include n = 1000 patients. Including centers submit information on clinicopathological variables, including local

pathology reports. Patient-reported outcomes and follow-up are self-registered by consenting patients and validated by study personnel.

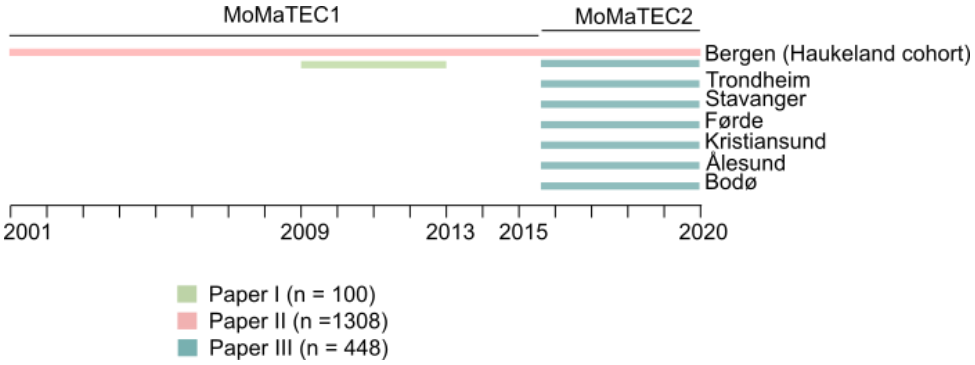

Figure 9. Patient series included in this thesis. MoMaTEC, Molecular markers in the treatment of endometrial cancer (study).

3.2 Analysis of biological tissue

3.2.1 Liquid chromatography/Tandem mass spectrometry (LC-MS/MS) of plasma hormone levels

For paper I we employed an LC-MS/MS panel already in clinical use, and therefore validated and available for easy implementation. The panel included progesterone, 17-hydroxyprogesterone, 11-deoxycortisol, cortisol, androstenedione and testosterone[167]. We also measured estrone and estradiol, using a novel sensitive LC-MS/MS protocol designed for quantification of these hormones in post-menopausal women[168] (Table 3). The referenced method-articles above describe the development of the methods in detail.

Preoperative blood samples were collected in Ethylenediaminetetraacetic acid (EDTA)-tubes, centrifuged at 1600 g for 15 minutes. Plasma was pipetted and stored at -80 °C. The hormone analyses were performed by the Hormone laboratory at Haukeland University Hospital.

Table 3. Limits of detection for the included LC-MS/MS analyses. Source: Methlie et al 2013[167] and Berthelsen et al 2020[168]

	Limit of Detection	**Lower limit of quantification***
17-hydroxyprogesterone (nmol/L)	< 0.06	0.24
11-deoxycortisol (nmol/L)	<0.03	0.10
Testosterone (nmol/L)	<0.01	0.02
Androstenedione (nmol/L)	<0.02	0.12
Progesterone (nmol/L)	<0.06	0.12
Estradiol (pmol/L)	<0.28	0.58
Estrone (pmol/L)	<0.15	0.25

* Lowest value where coefficient of variance ≤ 20%

LC-MS/MS is a technique for quantification of different chemical compounds within a sample. The sample is prepared in a liquid phase which passes through a chromatographic column under ultra-high pressure leading to a separation of the analytes based on their affinity to the respective phases (mobile/stationary). The temporarily resolved liquid phase compounds are ionized in an interface and separated according to mass to charge ratio in a mass spectrometer (Figure 10). In tandem mass spectrometer setups, additional steps of mass spectrometry allow for filtering out compounds and fragmenting the targeted compounds to increase the resolution.

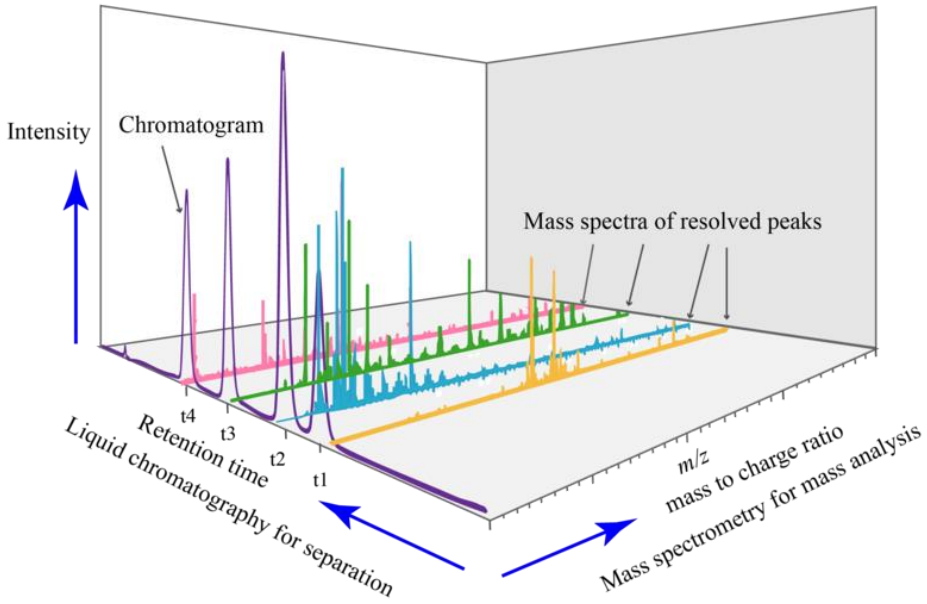

Figure 10. Resolution of compounds in LC-MS/MS related to retention time in chromatography column and mass to charge ratio. By Daniel Norena-Caro. Permission under CC0 1.0 Universal public domain dedication.

3.2.2 Immunohistochemistry

In paper I, immunohistochemical staining assessments from tissue micro-arrays (TMA) were available for expression of ER, PR, androgen receptor (AR) and glucocorticoid receptor (GR) from previous research projects and were used to explore associations to steroid levels [169-172]. In paper II, ER and PR expression in preoperative biopsies was assessed routinely in full sections at the Department of pathology, Haukeland University Hospital. Data on ER/PR expression was retrieved from the clinical pathology report.

Briefly the method employed at our lab for immunohistochemistry follows. Formalin-fixed paraffin-embedded tissue was sectioned in 5µm slides. Slides were dewaxed in xylene and hydrated in a stepwise ethanol gradient of decreasing concentration. Antigen retrieval was performed by microwave heating for 15 minutes in pH6 or pH9

buffer, followed by silencing of endogenous peroxidase with a blocking anti-peroxidase. Primary antibodies were added in specific dilutions and for a defined duration. The corresponding species-specific secondary antibody and the enVision DAB+ system (DAKO, Carpinteria, CA) were used to develop staining. Slides were dehydrated and mounted prior to microscopic evaluation. For specifics for each staining procedure, refer to the above referenced publications.

Slides were evaluated blinded for patient and tumor characteristics. A staining index was calculated as the product of staining intensity (0-3) and area of positive tumor cells (1: <10%, 2: 10%-50%, 3:>50%). Interobserver variability was assessed by two independent observers scoring random slides. Finally, the staining scores were dichotomized with consideration to survival characteristics, group sizes and number of events.

3.2.3 RNA microarray studies

In paper I, available microarray mRNA expression data from for 77 included patients with endometrioid histology was retrieved to assess patterns in gene expression in relation to levels of circulating sex hormones. A brief description of the method employed follows.

Samples for mRNA studies were collected during surgery, snap-frozen in liquid nitrogen and stored at -80 °C. Hematoxylin-stained frozen sections were assessed by light microscopy to determine tumor cell content prior to RNA extraction. Lesions with at least 50% and preferably 80% tumor cell content were selected. Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany) and quantified by the Nanodrop 1000 (Thermo Fischer Scientific, Waltham, USA). Integrity and quality were measured by the Bioanalyzer 2100 (Agilent, St Clara, USA). RNA was hybridized on Agilent Whole Genome Microarray 44k (Cat. No. G4112F, Agilent, St Clara, USA). Scanning was performed using the Agilent Microarray Scanner Bundle (Agilent, St Clara, USA). Expression data was quantile normalized and log2 transformed before gene expression analysis. J-express (Molmine, Bergen, Norway) was used to analyze

data, and significance analysis of microarrays (SAM) and gene set expression analysis (GSEA) were performed (Figure 11).

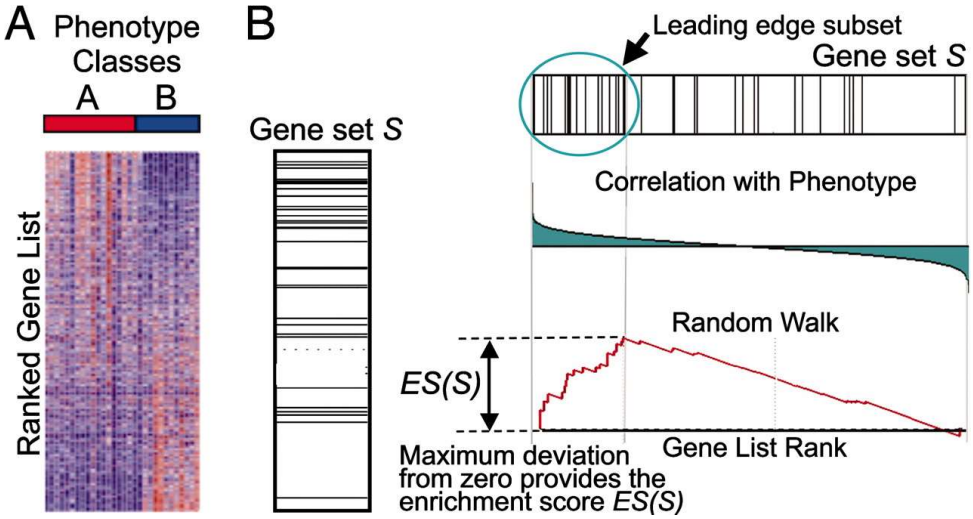

Figure 11. Gene set enrichment Analysis. A) Ranked heatmap of gene expression by phenotype, providing the ranked gene list. B) Gene set S providing the genes of interest. The algorithm "walks" down the ranked gene list increasing the enrichment score (ES) when encountering a gene in S and decreasing when no gene in S is encountered. Figure from Subramanian et al. 2005[173] with permission. Copyright (2005) National Academy of Sciences, U.S.A.

3.3 Imaging

3.3.1 Computer tomography and body fat distribution

Fat-segmentation data from CT scans, previously reported[174], was available for 83 patients in paper I.

Contrast-enhanced CT scans including thorax, abdomen and pelvis were obtained from the routine workup of the patients. The CT scans were assessed for abdominal fat distribution using iNtuition (TeraRecon Inc., San Mateo, CA, USA). The software provides a semi-automated volumetric segmentation in subcutaneous and visceral compartments based on Hounsfield units. The boundaries were controlled and adjusted by the operator when necessary.

3.3.2 Magnetic resonance imaging

MRI findings reported in the standard radiological report including myometrial invasion, cervical stroma invasion, and extrauterine disease were reviewed and recorded for paper II.

MRI was only sporadically used for evaluation in endometrial cancer until 2009 when pelvic contrast-enhanced MRI was routinely included in the preoperative work-up. The MRI examinations referred to in this thesis were performed at Haukeland University Hospital or at referring hospitals (varying brands and field strength, 1.5 or 3 Tesla). The imaging protocols largely adheres to European guidelines[175]. Protocol-adherent pelvic imaging included T2-weighted sagittal and axial oblique (transverse planes of corpus uteri) and axial T1-weighted gradient-echo images before and after intravenous contrast.

3.3.3 PET/CT

For paper II, PET/CT findings reported in the standard radiological report (FDG-avid lymph nodes or other sites) were reviewed and recorded.

18F-FDG PET/CT was included in the standard work-up of endometrial cancer patients from 2011. All scans were performed at Haukeland University Hospital on a Siemens Biograph 40 True Point or a Siemens biograph Vision scanner (the latter after November 2018), ranging from skull base to mid-thigh. During the study period, the associated CT protocol was changed from diagnostic to low-dose protocol.

3.4 Assessing quality of life and patient reported outcomes

For Paper III, PRO measures from MoMaTEC2 were analyzed. The questionnaires EORTC-C30 (general) and EORTC-EN24 (endometrial cancer specific) questionnaires were chosen for the MoMaTEC2 protocol as they are validated and available in translations for all participating countries (specimens in Appendix A). The EORTC questionnaires were answered at baseline (preoperatively) and annually postoperatively. The prospective registration allowed for adjustment for baseline properties of importance. Age, body mass index, comorbidity, tumor stage and marital

and socioeconomic status may be important predictors of PROs in endometrial cancer patients[176-178], and these can be approximated by including baseline PRO values.

The EORTC questionnaires are constructed of items, where each item requires the respondent to evaluate the item of interest over the last week (with exception for "sexual activity" and "sexual interest" which is evaluated for the previous 4 weeks). The item responses are Likert scales ranged 1-4 from "None", through "A little" and "Quite a bit" to "Very much". The Global health score/Quality of life item responses are ranged 1-7. For analysis, the EORTC recommends transforming item responses to scales through grouping of related items and normalizing according to the EORTC scoring manual[179], thus producing 15 scales for the EORTC-C30 and 13 Scales for the EORTC-EN24, ranging from 0-100 (Table 4). These scale scores are discrete, taking values depending on the number of items and item ranges included.

Table 4. Scales included in the European organization for research and treatment in cancer EORTC questionnaires c30 (general) and EN24 (endometrial cancer specific)

Abbreviation	Name	Type*	Number of Items	Explored in paper III
EORTC-C30				
QL2	Global health status/QoL	Function	2	x
PF2	Physical Function	Function	5	x
RF2	Role Function	Function	2	
EF	Emotional Function	Function	4	x
CF	Cognitive Function	Function	2	x
SF	Social Function	Function	2	x
FA	Fatigue	Symptom	3	x
NV	Nausea / vomiting	Symptom	2	
PA	Pain	Symptom	2	
DY	Dyspnea	Symptom	1	
SL	Insomnia	Symptom	1	
AP	Appetite loss	Symptom	1	
CO	Constipation	Symptom	1	
DI	Diarrhea	Symptom	1	
FI	Financial problems	Symptom	1	
EORTC-EN24				
ENLY	Lymphoedema	Symptom	2	x
ENUR	Urological symptoms	Symptom	4	x
ENGI	Gastrointestinal symptoms	Symptom	5	x
ENBI	Poor body image	Symptom	2	x
ENSXV	Sexual/vaginal problems	Symptom	3	x
ENBP	Pain in back and pelvis	Symptom	1	x
ENTN	Tingling/numbness	Symptom	1	x
ENMP	Muscular pain	Symptom	1	x
ENHL	Hair loss	Symptom	1	x
ENTC	Taste change	Symptom	1	x
ENSXI	Sexual interest	Function	1	x
ENSXA	Sexual activity	Function	1	x
ENSXE	Sexual enjoyment	Function	1	x

* Function scales increase with increasing function (improvement), symptom scales increase with increasing level of symptom (deterioration)

3.5 Statistical methods

All statistical analysis was performed in Statistical Program for the Social Sciences version 25 (SPSS, IBM inc., Chicago, IL, USA) and R version 3.6.1-4.0.2 (R Core Team 2020) R Foundation for Statistical Computing, Vienna Austria). For comparison of categorical data, the Chi-square test was preferred, but Fischer exact test performed when included frequencies were ≤5. No continuous data variables analyzed in these projects were normally distributed, and Mann-Whitney U test was used to compare distributions between 2 groups and Kruskal-Wallis test between 3 or more.

Trends in changes of clinicopathological characteristics over time were analyzed by linear regression in the case of continuous variables (age, body mass index) and trends of proportions by the Chi square test for trend. To determine cutoffs for the prediction of 5-year disease-specific survival by levels of endogenous steroids, Receiver Operating Characteristics (ROC) curves were generated. Area under the curve was analyzed to establish the analytic accuracy and Youden index used to select cutoff.

Overall survival was defined as time from treatment to death from any cause. Disease-specific survival was defined as time from treatment to death from endometrial cancer. Recurrence-free survival was defined as time from surgery to first verified recurrence, and only included patients with completely resected tumors (macroscopically tumor-free). Survival statistics were visualized in Kaplan-Meyer curves and differences between groups calculated by the log-rank test. Cox proportional hazard regression was used to perform multivariable survival analysis.

For analysis of PROs and quality of life endpoints, linear mixed models were used. For each EORTC scale a model was fitted, using the scale score as outcome variable and treatment modalities (laparoscopy/laparotomy, lymph node staging procedure, adjuvant chemotherapy) as predictors (fixed effects). A patient-level random intercept was included as well as a baseline covariate and interactions for time and treatment.

Two-sided P-values < 0.05 were considered significant.

4. Summary of results

Paper I

We evaluated steroid hormone levels in blood samples drawn from 100 postmenopausal patients, selected to match histological and patient-related characteristics of the whole population-based cohort (Haukeland cohort). We analyzed a panel of sex-hormone related steroids, routinely used in the clinic to diagnose endocrinological conditions, in addition to a novel estrogen assay developed to quantify post-menopausal levels of estrogen. To assess the biomarker potential of these assays in endometrial cancer we explored associations with immunohistochemical expression, gene expression, radiologic body-composition parameters and survival. We found that low levels of 17-hydroxyprogesterone, 11-deoxycortisol and androstenedione were associated with aggressive tumor characteristics and poor disease-specific survival. 17-hydroxyprogesterone and 11-deoxycortisol both predicted outcome independently of preoperatively assessed histological type and grade in multivariable analysis. Tumors from patients with low levels of these hormones expressed gene sets correlating to mitosis and cell-cycle progression to a higher degree and inflammatory and estrogen-signaling gene sets to a lower degree than those with high levels. Levels of estrone and estradiol were associated with transcriptional estrogen signaling, expression of hormone receptors and higher measurements of body fat, but not to survival.

Paper II

We reviewed all endometrial cancer patients receiving primary treatment at Haukeland University Hospital over the period 2001-2019 with a focus on comparing outcomes before and after implementing treatment changes. Main treatment changes were discontinuation of radiotherapy as an adjuvant treatment from 2009 and a transition to a biomarker- and imaging-based selective lymphadenectomy policy in 2012-2013 (to replace a systematic lymph node sampling policy). Stage III patients treated in the post-2009 period had better overall survival (5- year OS 0.61 vs 0.49, $p = 0.04$), disease-specific survival (5-year DSS 0.68 vs 0.54, $p = 0.06$ and recurrence-free survival (3-

year RFS 0.71 vs. 0.51, p = 0.03). No differences in survival were found in other stages. There were no significant changes in total recurrences or recurrences by site between patients treated before 2009 and after. A marked reduction in the total number of lymphadenectomies after 2012 resulted in an increase in the group of patients classified as early-stage disease but with unknown nodal status, and recurrence-free survival in this group was maintained compared to non-lymphadenectomized early-stage patients treated 2001-2012. Also of note, there was a substantial increase in adjuvant therapy given to stage I high-risk patients after 2009, without any corresponding improvement in survival.

Paper III

In this study we determined the effect of treatment modalities on patient-reported outcomes during the first post-operative years. We analyzed prospectively collected data on symptoms, function, and quality of life among Norwegian patients enrolled in MoMaTEC2. We found overall good quality of life and functional outcomes compared to reference population means at one and two years, but lower means at baseline. Patients treated with chemotherapy reported more peripheral neuropathy, lymphedema and muscular pain at follow-up. Among patients not receiving chemotherapy, lymph node staging procedures were not associated with worse symptoms. In multivariable mixed models, adjuvant chemotherapy increased peripheral neuropathy, lymphedema, fatigue, and reduced physical functioning. There were no independent effects of lymph node staging on symptoms.

5. Discussion

5.1 Methodological considerations

5.1.1 Strength and weakness of study design

Randomized controlled trials (RCT) are considered the gold standard when settling clinical research questions in medicine. Properly conducted, they eliminate as much bias as possible, and provide evidence for cause-effect relationships. They are however cumbersome in nature and place high demands on pre-trial planning, participating personnel, patients, and follow-up structure in order to be successful[180]. A problem when interpreting RCTs is low power leading to high risk of random sampling error, reflected in high variability of the outcome statistic (inaccuracy)[181]. There is also concern that in optimizing the cohort to answer a specific question, relevant subgroups may be excluded, since results are only valid for the group represented by the participants[182]. In contrast, observational studies are easier to perform, and can provide data on associations between treatment and outcome but will be biased due to non-randomization[183]. In many instances a definitive RCT will be necessary, especially when available evidence has important implications but is conflicting. Often however, because of economy, unattainable power estimates, impossibility to randomize, or other reasons, data from observational studies will be the ultimate basis for conclusions.

All of the papers included in this thesis are observational, and methodological considerations regarding study design and sources of bias will be briefly discussed below.

For paper I, a selection of patients was performed to represent a population-based cohort. The advantage of using a population-based cohort is that the results are more robust to variability among patients and therefore are more likely to represent the population, thereby reducing the chance of selection bias. In paper I, the objective was to identify novel biomarkers, and the cohort served as a hypothesis cohort. As findings from such a cohort may be biased by multiple testing issues and random sampling

effects, any results need to be confirmed in an independent validation cohort before being considered for clinical use.

Paper II was a population-based cohort study investigating the effects of changing therapy regimens at a large tertiary hospital. Findings related to differences in time periods are difficult to interpret as there are several simultaneous changes over the observation period that could affect outcome. To partly account for this, an analysis of relevant risk factors over time was performed. Bias resulting from overall changes in treatment approach (more therapy in total, different surgeon approach to selecting treatment) was however likely to be present. In contrast, a strength of Paper II is that it determined the effect of changing treatment under conditions that exist in the ordinary clinical setting. “Real world” research is valuable and necessary as it often moderates overly optimistic results from RCTs and can give more accurate ideas of cost-effectiveness of implementing changes. The observations made in paper II can support data from relevant RCTs and raise questions that should be further explored in RCTs.

Paper III was a cohort study derived from the ongoing MoMaTEC2 project. For Paper III, PRO questionnaires collected up to a specified time-point (November 2020) were analyzed in relation to treatment received. As patients are not randomized to treatment, results may be biased by grouping of patients with confounding characteristics such as socioeconomical or health-related factors that could have bearings on the PROs. Including pretreatment baseline values, that represent some of the variation caused by non-treatment related factors, provide some compensation[184]. Ideally, an RCT would need to be performed with randomization to treatment to settle causality. An observational study is however suited to uncover associations between treatment modalities and PROs and can pinpoint targets for further focused research.

5.1.2 Considerations regarding biological analyzes

In paper I multiple methods were employed to describe various properties of the patient and tumor. LC-MS/MS was used to quantify steroid hormones in plasma, blood samples were submitted to be tested by routine clinical protocols. LC-MS/MS is considered the gold standard for analysis of steroid hormones in human plasma and is

in wide-spread clinical use. High cost and low throughput may be mentioned as drawbacks compared to earlier methods such as immunoassays, but this is generally weighed up by excellent specificity, resolution, and multiplexing possibilities[185].

Issues can be raised on the representativeness of frozen samples. Previous studies report little degradation of steroid hormones in normal storage temperatures, and a generally unaltered ranking of samples over time[186, 187]. In addition, circadian cyclicity of steroid hormones is a bias that was not possible to account for due to the retrospective nature of the study. As the samples were collected simultaneously with the routine preoperative blood samples it is likely that the sample time points are evenly distributed between groups. This would need to be verified in a validating study with fixed sampling timepoints. We avoided issues with menstrual cycle variations by limiting the study to postmenopausal women, thereby targeting the majority of patients. To assess levels of these hormones in premenopausal women, samples would need to be taken on specific cycle days which may come in conflict with need for rapid treatment or at least be more demanding for included patients. This might however be warranted in future studies as conservative (fertility-sparing) treatment more often is an issue for this group, with a lack of prognostic biomarkers to guide treatment decisions.

Regarding inferences with mRNA expression, there are some concerns. Firstly, use of microarrays may not pick up all facets, as the preformed probes will not distinguish between isoforms, splice variants and similar post-transcriptional alterations unless pre-specified. Secondly, using mRNA as a surrogate for their end-product proteins is not optimal as mRNA expression only explains around 40% of the protein level variations[188], the rest being affected by post transcriptional regulation. The correlation may however be higher for certain groups of genes, as is suggested to be the case for genes differentially expressed between tumor subsets exposed to different treatment[189]. Also, translation is not the only important endpoint for mRNA, and transcriptomics may provide other important information on ongoing processes in the cell.

5.1.3 Considerations on measuring patient-reported outcome

In paper III, PROs were investigated in relation to treatment modalities. PRO assessment is characterized by diverse tools, methods of analysis, and interpretation, and methodological choices for this paper require a deeper discussion[190].

The tools and timing of PRO assessment depends highly on the aims of the study. Symptoms and quality of life during treatment with different adjuvant modalities are important when assessing which treatment should be preferred but may be different from long-term outcomes. The patient may prefer worse outcomes briefly during treatment to gain survival or avoid other long-term side effects. In addition, different tools may have different aspects that are important for the pursued aims such as documented reference values, validated translations or may have been used in other research were comparison is warranted.

In oncologic research, two tools for PRO assessment dominate: the Functional assessment of cancer therapy (FACT-G) and European Organisation for Research and Treatment of Cancer (EORTC) (table 5). Both contain numerous modules to adapt to different cancer settings and diagnoses and are rigorously validated. Comparisons show that psychometric properties are similar, there are however small differences that may be of interest when choosing tool for a trial or project[191].

Scales measuring quality of life and symptoms may be subjected to floor and ceiling effects. Floor effects are when many respondents report the lowest possible score, such as for a symptom that is not frequently present. Ceiling effects are when many respondents report the highest score, such as for functioning or quality of life, when these are not impaired in most patients. Both effects will result in non-normal distributions and standard deviations that span outside the scale limits. Floor and ceiling effects are shown to be present when using the EORTC-QLQ-C30 questionnaire[192] and are likely to be present for any scale depending on the population tested.

Table 5. Examples of common patient-reported outcome tools

Abbreviation	Name	Items general health	Items with EC specific module	PubMed (Cancer)*	PubMed (EC)*
FACT	Functional assessment of cancer therapy	27	43	7351	47
EORTC	European Organisation for Research and Treatment of Cancer	30	54	2921	24
SF-36	Short form	36	-	584	7
PROMIS	Patient Reported Outcomes Measurement Information system	10	-	315	0

EC: Endometrial cancer

* Number of hits with search in Pubmed restricted to 2017-2019 with query: [Abbreviation (e.g. "FACT")] AND [keyword (cancer or EC)]

Despite non-normal distribution, parametric indices (mean, standard deviation) are commonly applied, and likely more informative than medians and percentiles. We opted for parametric indices for descriptive purposes and comparison with previous research. Non-parametric hypothesis testing was performed in paper III, although parametric methods are likely equally appropriate with large sample sizes.

Another challenge when analyzing PRO is determining the clinical meaning of results. For example, the meaning of a 10-point increase in "social functioning" on a scale 0-100 may not be evident. This problem may be approached in different ways. For use in clinical settings, defining thresholds that signify where a score becomes a potential health problem is a reasonable approach[193]. For research purposes, minimally important changes may be defined, optimally by comparing questionnaire output to independent measurements of the same entity, such as another questionnaire (anchor-based). This has been performed for the EORTC-QLQ-C30, but not for the EN24 questionnaire[194, 195]. Osoba and colleagues suggested that EORTC-QLQ-C30 changes between 10-20 points represented (clinically) moderate changes, by using anchoring questionnaires[195]. Another approach is to use distributions. Cohens d (effect size) is commonly used, which is simply calculating mean/standard deviation in the sample[196]. An effect size of 0.5 has been shown to correspond to clinically meaningful changes[197].

It is also important to be aware that clinical meaningfulness can vary according to factors such as age, culture or disease status and should be qualitatively evaluated for each situation.

In analysis of PROs and quality of life-endpoints, many different statistical approaches may be justified. The Setting International Standards in Analyzing Patient-Reported Outcomes and Quality of Life Endpoints (SISAQOL) consortium has attempted (in an ongoing effort) to systematize and recommend specific analyzes depending on the aims of the study[190].

For longitudinal assessment of PROs over multiple timepoints, use of linear mixed models are suitable, and recommended by SISAQOL[190]. Linear mixed models are essentially linear regression models that can account for different levels of dependence between individual datapoints (also called hierarchical structures), such as repeated measurements over time in the same subject[198]. Linear mixed models offer advantages over other statistical models for longitudinal data:

1. Allows for different intercepts and/or different slopes for different hierarchical levels; each patient can have its own y-axis intercept for an outcome, which is biologically plausible in most situations.
2. Allows for retaining cases despite missing values. As an example, ANOVA is restricted to complete case analysis, meaning that all data points for a subject will be disregarded if one point is missing. (Non-random missingness will naturally still bias results in mixed models)
3. Effects of predictors on outcome can be analyzed at different time-points or can be averaged over the whole period according to hypothesis.

5.2 Discussion of results

5.2.1 Peroperative lymph staging and adjuvant treatment are key factors to improve endometrial cancer treatment

Limiting the number of patients who undergo staging procedures and/or adjuvant treatment is logical and effective but depends on accurate biomarkers. In this thesis, contributions to such an optimization have been made within different areas of endometrial cancer treatment.

In Paper II, we showed that the implementation of a risk-based algorithm to reduce the number of lymphadenectomies maintained the rate of detected metastasis and did not worsen survival for patients where the procedure was omitted. This shows that implementation of selective lymphadenectomy strategies is likely effective, and opens for tailored algorithms applying available biomarkers. In Paper I, a potential improvement to such a strategy was explored. High plasma levels of 17-hydroxyprogesterone and 11-deoxycortisol were found to be associated with better survival, independent of the prognostic information gained from histologic typing and grading.

In Paper III, focus was moved from survival to patient-reported outcomes. We determined what parameters of treatment had the greatest impact on the health-related quality of life of endometrial cancer survivors. A selective lymphadenectomy policy and the outcome of surgical staging determined the level of treatment in these patients. Outcomes differed negatively for patients receiving the most treatment. These results further stressed the need to reduce the treatment burden where it is not overtly necessary to improve function, symptoms, and quality of life. Taken together, this thesis highlights different areas where improvements to endometrial cancer treatment can be achieved and how patients may be affected by these changes.

5.2.2 Circulating sex steroid levels are associated with aggressive tumor traits and poor survival

We demonstrated associations with several circulating sex steroids and prognosis in endometrial cancer patients (Paper I). The steroids 17-hydroxyprogesterone, 11-

deoxycortisol and androstenedione were all negatively associated with poor outcome. In the synthesis pathway, these hormones are direct metabolites of each other, without any significant rate-limiting step (Figure 7). Although only 17- hydroxyprogesterone and 11-deoxycortisol were prognostic independent of histology it is not unlikely that the properties of these hormones are connected and in essence represent the same biomarker mechanism. To our knowledge, these steroids are not previously implicated as biomarkers in cancer (with the possible exception of rare adrenal and ovarian hormone-producing tumors[199]). Androstenedione has been shown to be elevated in patients with type I tumors compared to type II[31] and is associated with endometrial cancer risk in epidemiological studies[21, 33, 38, 126], but the significance of varying levels for prognosis has not previously been reported. 17-hydroxyprogesterone is not to a large degree converted into androgens in humans under normal conditions, but is implied to be a marker for increased ovarian production of androgens in women with polycystic ovary syndrome[200, 201]. A previous study from our group, comparing steroid levels in 19 patients with good survival outcome to 19 patients with poor survival identified other related hormones as promising biomarkers, none of which were available in our "clinical" panel[127]. However, for both 17- hydroxyprogesterone and 11-deoxycortisol, levels were higher for the good survival group, although not to the level of statistical significance.

The role of these endogenous intermediate steroids in post-menopausal women is not well known. In our study, higher levels of several of the steroids were associated with expression of progesterone receptors, implying that a higher proportion of these tumors exhibit active estrogen signaling. Theoretically, levels of steroids could reflect a host phenotype that affects what subtype of endometrial cancer is most likely to develop. In endometrial cancer, such a connection is established between obesity and endometrioid tumors, via estrogen levels[39], but we could not identify any correlation between obesity or fat distribution and the prognostic hormones. Thus, explanations of individual differences in steroid levels may be sought elsewhere, such as ovarian rest function (preoperatively) or overall adrenal function. There is also an emerging body of literature to support an important role for intracrinology in endometrial cancer, where

hormonal precursors can be locally metabolized (in/around the tumour) into active compounds (e.g. estrogens) that the tumor can utilize[131, 132, 202, 203]. Interestingly, in ovarian cancer, differences in the expression of stromal steroid metabolizing enzymes in different histological subtypes have been found[204]. Together with these findings, our study supports that other steroid hormones than the estrogens and progesterone can play important roles in endometrial tumorigenesis.

We show that biomarker data to improve stratification of patients on basis of prognosis may already be available in the clinic. As blood samples are already collected as part of the general workup, no extra invasive procedures are required. Although the biomarker properties of the sex steroid precursors have not previously been recognized, other circulating biomarkers have been proposed. Most notably, CA-125 has gained much attention due to its role in the diagnosis and follow-up of gynecological malignancies and is included in multivariable risk scores to select patients for lymphadenectomy[75, 76]. Adding additional blood sample parameters to these tools would not increase invasiveness and could improve stratification performance. Low 17-hydroxyprogesterone was associated with aggressive tumor characteristics within the endometrioid subgroup, pointing to a role in further stratifying low-risk patients with regard to treatment decisions.

In our institution, ER and PR immunohistochemistry analyzes in preoperative biopsies (curettage/pipelle) are utilized together with MRI and PET/CT imaging to select patients for lymphadenectomy, and the implementation of blood biomarkers could be added to refine stratification. However, further validation of these blood steroids in an independent cohort and an assessment of its predictive value for lymph node metastasis detection is needed before implementation can be considered.

5.2.3 Lymph node staging can be limited to certain risk groups

Reducing morbidity induced by lymphadenectomy has been a long-standing goal in endometrial cancer treatment. Sentinel node biopsy is a promising alternative to traditional lymphadenectomy but requires procedure experience for best performance[91]. In Norway, low-risk endometrial cancer has generally been treated at

secondary centers, with the benefit of keeping treatment and follow-up connected and close to home. To implement a state-of-the-art sentinel node program would require tertiary hospitals to accommodate more patients, and a loss of hysterectomy volume would ensue at local hospitals. Alternatively, sentinel node biopsy could be avoided in patients at low risk (determined by biomarkers) and be performed in high-risk patients at tertiary centers. This would possibly maintain necessary competence at the different levels of the health care institutions.

As discussed earlier, comprehensive removal of lymph nodes is not considered to improve survival[82, 83], and although it has important bearings on adjuvant treatment, there may be room for strategies that choose observation in node-agnostic patients and treat when recurrence is overt. We demonstrated that when applying a selective lymphadenectomy algorithm based on imaging findings and preoperative assessment of ER/PR expression, some recurrences will occur among non-staged patients, but with comparable frequency to those that were node negative (Paper II). Other groups have published performance data on promising selective lymphadenectomy algorithms[75, 76, 205]. Our study provides data from a population-based setting showing efficient reduction of lymphadenectomy procedures without reduction in survival, supporting a selective lymphadenectomy strategy. We are not aware of any other studies that compare survival outcomes between patients undergoing systematic versus selective lymphadenectomy.

Current literature supports equal survival outcomes for lymphadenectomy and sentinel node techniques[93, 206, 207]. Although the risk for lymphedema and related complications is likely lower for sentinel node biopsy than for conventional lymphadenectomy[89], the absolute difference is not known, especially when radiotherapy is not used[208]. Among patients in our study (Paper III), lymphedema was more strongly associated with chemotherapy than lymph node staging. This effect remained when grouping sentinel node biopsy with no lymphadenectomy and comparing to more comprehensive lymphadenectomy techniques. Thus, the effect of lymphadenectomy on lymphedema when adjuvant radiotherapy is omitted may be overrated.

A randomized trial comparing sentinel node biopsy to selective lymphadenectomy is unlikely as surgical techniques are inherently difficult to randomize (due to factors such as demands for surgical experience, surgeon preferences, equipment availability etc.) and the necessary number of participants would have to be high as complications are rare. When considering results from paper II and III, selective lymphadenectomy appears to be a viable alternative to sentinel node biopsy, and perhaps more relevant for the Norwegian health care system and countries with similar demographics. MoMaTEC2 is expected to provide important data on implementing selective lymphadenectomy, compared to systematic lymphadenectomy, and also compared to centers performing sentinel node biopsy.

5.2.4 Optimizing adjuvant treatment is vital to improve quality of life for endometrial cancer patients

The gap between preclinical progress in uncovering new potential treatment mechanisms and clinically implemented therapy is huge. Few endometrial cancer patients are offered targeted treatment. Instead, traditional adjuvant treatment, either chemotherapy or radiotherapy, is standard of care to reduce the risk of recurrence. As the majority of these patients will survive without recurrence, any treatment-specific complications or morbidity will weigh negatively on the treatment decision scale. Also, many patients in stage III will recur in spite of adjuvant treatment[101, 102], with new treatment morbidity added to the previous in a cumulative fashion, where the utility of the primary adjuvant treatment may be questioned. Recently, registration of patient-reported outcomes has come more into focus. Data collected from PRO studies is useful to aid patients participating in treatment planning, to make physicians aware of new problems during follow-up and to direct research into areas where care can be improved[150]. We demonstrated how endometrial cancer patients rate their health-related quality of life and treatment-related symptoms (Paper III). We showed that at the time of diagnosis, quality of life and emotional functioning is low, but improves after treatment. This can be explained by the baseline data being collected at a time point where the patients have recently been handed a cancer diagnosis, without final prognostic information and with possible bleeding symptoms/pain and apprehension towards surgery added. Similar results are found in other longitudinal studies[160, 176, 177].

Availability of healthy-population reference data was a strength of this study and allowed for better interpretation of results. In contrast, a mean increase in self-reported lymphedema and peripheral neuropathy (tingling/numbness) can be seen after treatment. We found these changes to be concentrated to the group receiving the most treatment, and multivariate analysis implicated adjuvant chemotherapy as the most relevant factor. In addition to the above-mentioned symptoms, chemotherapy was associated with increased fatigue and decreased physical function. In patients not receiving adjuvant chemotherapy, we observed no differences in reported symptoms in relation to lymph node staging. As rates of adjuvant radiotherapy are very low in our population (due to national guidelines) we could not investigate its effect on these outcomes. MoMaTEC2 includes patients from international sites where the use of post-operative radiation is routine and will hopefully provide further insight.

There is likely potential in refining algorithms for adjuvant treatment. In paper II we found that in spite of a substantial increase of chemotherapy in the treatment of early-stage high-risk patients, no differences in survival could be seen. A Danish multicenter study (ClinicalTrials.gov Identifier: NCT1244789) is prospectively investigating the randomization of these patients to adjuvant chemotherapy or observation, and results are expected in a couple of years. The most recent European guidelines are incorporating molecular subtypes in addition to uterine factors to assess the need for adjuvant therapy[66], and studies are ongoing to determine if one can refrain from adjuvant treatment in the presence of POLE-mutations (the RAINBO-study) or other risk factor combinations (the PORTEC-4a-study). These studies are mainly performed at centers with liberal adjuvant radiotherapy policies and need to be complemented by similar studies performed in regions using primarily chemotherapy.

5.2.5 Obstacles for the clinical implementation of biomarkers

In the search to improve patient treatment, preclinical research is a vital foundation. The establishment of the Bergen biobank has led to a formidable opportunity to discover new biomarkers such as ER/PR expression or circulating steroid hormones. Further development of these markers into clinical tools is however cumbersome. MoMaTEC2 is assessing the implementation of ER and PR immunohistochemically

assessed in preoperative biopsies as biomarkers into clinical practice and will provide important information on this process. Combining several biomarkers across disciplines is likely necessary to achieve good enough performance to add new value to clinical decisions. In the event that an optimal algorithm emerges, there are further challenges before implementation can be realized. Invasiveness, cost and reliability can and should be incorporated when nominating new biomarkers and can affect how the biomarker or algorithm performs in the clinic[209]. In addition, real-world challenges such as obesity or frailty can alter treatment decisions based on biomarkers[210-213]. In paper II, we found that around 50% of low-and intermediate risk patients that underwent lymphadenectomy did not meet algorithm criteria but were staged at the surgeon's discretion. Interestingly, none of these patients had positive lymph nodes. This can be interpreted in two ways; either the algorithm has potential for an even more discriminative selection, or the clinical reality does not allow for pure algorithmic decision making. Likely the truth is a place in between, which highlights the importance of evaluation of implementation in real-world conditions.

6. Conclusions

Paper I: Blood steroids have prognostic properties in endometrial cancer. They provide preoperative information independent of the histological subtype and grade and are easily available biomarkers.

Paper II: A selective lymphadenectomy algorithm based on preoperative histological and radiological biomarkers reduced the rate of lymphadenectomy from approximately 80% to 50%, while maintaining good survival.

Paper II: Omitting adjuvant radiotherapy and implementing an adjuvant chemotherapy alone policy was followed by maintained survival for the whole cohort, with improved survival for FIGO stage III patients. Despite a substantial increase in administration of adjuvant chemotherapy in early-stage high-risk patients, there was no gain in recurrence-free survival for this group.

Paper III: Overall quality of life and function is good in endometrial cancer survivors, however mean increases in lymphedema and neuropathy symptoms are reported. Patients receiving adjuvant chemotherapy perceive substantially increased neuropathy at years one and two, and increased lymphedema at year one. Patients that undergo lymph node staging but do not receive adjuvant therapy do not differ from patients that are unstaged regarding PRO at one and two years after treatment.

7. Future aspects

Improving endometrial cancer treatment is vital, and in this pursuit two main obstacles can be identified. A lack of effective treatment options for advanced disease and recurrence, and over-treatment or too indiscriminative treatment for presumably localized disease. To improve survival for advanced stage endometrial cancer, it is likely we need new therapeutic agents and better tailoring of treatment to individual patients and tumors. To reduce the treatment burden for patients with early-stage disease we need to better identify those at risk for recurrence and stratify patients by effect of adjuvant treatment. Chemotherapy and/or radiotherapy is still likely to play a major role in endometrial cancer treatment in the years to come, and predictive biomarkers to assess the potential effect of these treatments are of vital importance. A better mapping of quality of life and PRO effects with regard to reductions in treatment burden is needed, as well as direct comparisons between treatment options with high-quality PRO registration, to ensure that treatment-related morbidity is minimized.

More specifically, to push forward from the results presented in this thesis the following research is needed.

Accurate measurement of circulating sex steroids is available in the clinic and prognostic in endometrial cancer. The prognostic potential needs to be validated in an independent cohort. Secondly, the best use of this biomarker needs to be determined, likely through combination with other prognostic biomarkers. Also, the predictive capabilities of circulating steroids regarding hormonal and fertility-sparing treatment should be explored.

Sentinel node biopsy is on the rise, and is likely to replace lymphadenectomy in the staging of endometrial cancer. High-quality evidence of improvements in long-term quality of life and treatment-related symptoms are needed to motivate this transition. Selective sentinel node algorithms can be an alternative to reduce cost, maintain treatment logistics and avoid transition of patient volumes from secondary to tertiary centers. An RCT to compare a selective sentinel node strategy to the current state-of-the-art sentinel node (for all) strategy should be conducted. Observational studies

comparing these strategies between centers, such as MoMaTEC2, will provide important data in the absence of an RCT.

The potential of reducing chemotherapy in early-stage high-risk patients needs to be further explored. Identification of new biomarkers and better staging could lead to better identification of patients truly at risk for recurrence. An intensified search for biomarkers that predict effects on recurrence by platinum-based chemotherapy could isolate the group that has a survival effect to balance out morbidity and indicate where other therapies such as immune therapy should be tried out. Most pressingly, an assessment of the TCGA molecular classes with regard to chemotherapy effectiveness needs to be conducted.

The results of Paper III point to important adverse effects of chemotherapy present over years. Clinical PRO measurement can be implemented in patient follow-up and should be monitored and analyzed to assess how this may affect self-perceived quality-of-life and symptoms in women that survive endometrial cancer.

8. References

1. SUNG H, FERLAY J, SIEGEL RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians 2021;n/a.
2. CANCER REGISTRY OF NORWAY. Cancer in Norway 2019 - Cancer incidence, mortality, survival and prevalence in Norway. Oslo: Cancer Registry of Norway, 2020.
3. FABER MT, FREDERIKSEN K, JENSEN A, AARSLEV PB, KJAER SK. Time trends in the incidence of hysterectomy-corrected overall, type 1 and type 2 endometrial cancer in Denmark 1978–2014. Gynecologic oncology 2017;146:359-67.
4. CLARKE MA, DEVESA SS, HARVEY SV, WENTZENSEN N. Hysterectomy-Corrected Uterine Corpus Cancer Incidence Trends and Differences in Relative Survival Reveal Racial Disparities and Rising Rates of Nonendometrioid Cancers. J Clin Oncol 2019;37:1895-908.
5. SHEIKH MA, ALTHOUSE AD, FREESE KE, et al. USA endometrial cancer projections to 2030: should we be concerned? Future Oncol 2014;10:2561-8.
6. KURNIT KC, WARD KK, MCHALE MT, SAENZ CC, PLAXE SC. Increased prevalence of comorbid conditions in women with uterine cancer. Gynecologic oncology 2015;138:731-34.
7. BOKHMAN JV. Two pathogenetic types of endometrial carcinoma. Gynecologic oncology 1983;15:10-7.
8. SETIAWAN VW, YANG HP, PIKE MC, et al. Type I and II endometrial cancers: have they different risk factors? Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2013;31:2607-18.
9. REED BGC, B.R. The Normal Menstrual Cycle and the Control of Ovulation. In: Feingold KR AB, Boyce A, et al., editors., ed. *Endotext [Internet]*. South Dartmouth (MA): : MDText.com, Inc., 2018.
10. LU KH, BROADDUS RR. Endometrial Cancer. N Engl J Med 2020;383:2053-64.
11. RYAN NAJ, GLAIRE MA, BLAKE D, CABRERA-DANDY M, EVANS DG, CROSBIE EJ. The proportion of endometrial cancers associated with Lynch syndrome: a systematic review of the literature and meta-analysis. Genetics in Medicine 2019;21:2167-80.
12. YEHIA L, KEEL E, ENG C. The Clinical Spectrum of PTEN Mutations. Annual Review of Medicine 2020;71:103-16.
13. WIN AK, REECE JC, RYAN S. Family History and Risk of Endometrial Cancer: A Systematic Review and Meta-analysis. Obstetrics & Gynecology 2015;125.
14. BJORGE T, ENGELAND A, TRETLI S, WEIDERPASS E. Body size in relation to cancer of the uterine corpus in 1 million Norwegian women. International journal of cancer 2007;120:378-83.

15. BHASKARAN K, DOUGLAS I, FORBES H, DOS-SANTOS-SILVA I, LEON DA, SMEETH L. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5·24 million UK adults. The Lancet 2014;384:755-65.
16. KARAGEORGI S, HANKINSON SE, KRAFT P, DE VIVO I. Reproductive factors and postmenopausal hormone use in relation to endometrial cancer risk in the Nurses' Health Study cohort 1976-2004. International journal of cancer 2010;126:208-16.
17. HUSBY A, WOHLFAHRT J, MELBYE M. Pregnancy duration and endometrial cancer risk: nationwide cohort study. Bmj 2019;366:l4693.
18. MCPHERSON CP, SELLERS TA, POTTER JD, BOSTICK RM, FOLSOM AR. Reproductive factors and risk of endometrial cancer. The Iowa Women's Health Study. Am J Epidemiol 1996;143:1195-202.
19. LØCHEN ML, LUND E. Childbearing and mortality from cancer of the corpus uteri. Acta Obstet Gynecol Scand 1997;76:373-7.
20. BRINTON LA, TRABERT B, ANDERSON GL, et al. Serum Estrogens and Estrogen Metabolites and Endometrial Cancer Risk among Postmenopausal Women. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2016;25:1081-89.
21. LUKANOVA A, LUNDIN E, MICHELI A, et al. Circulating levels of sex steroid hormones and risk of endometrial cancer in postmenopausal women. International journal of cancer 2004;108:425-32.
22. BERGMAN L, BEELEN MLR, GALLEE MPW, HOLLEMA H, BENRAADT J, VAN LEEUWEN FE. Risk and prognosis of endometrial cancer after tamoxifen for breast cancer. The Lancet 2000;356:881-87.
23. BERAL V, BULL D, REEVES G. Endometrial cancer and hormone-replacement therapy in the Million Women Study. Lancet (London, England) 2005;365:1543-51.
24. ZHOU B, YANG L, SUN Q, et al. Cigarette smoking and the risk of endometrial cancer: a meta-analysis. Am J Med 2008;121:501-08.e3.
25. COLLABORATIVE GROUP ON EPIDEMIOLOGICAL STUDIES ON ENDOMETRIAL CANCER. Endometrial cancer and oral contraceptives: an individual participant meta-analysis of 27 276 women with endometrial cancer from 36 epidemiological studies. The Lancet Oncology 2015;16:1061-70.
26. IVERSEN L, SIVASUBRAMANIAM S, LEE AJ, FIELDING S, HANNAFORD PC. Lifetime cancer risk and combined oral contraceptives: the Royal College of General Practitioners' Oral Contraception Study. American journal of obstetrics and gynecology 2017;216:580.e1-80.e9.
27. PARAZZINI F, NEGRI E, LA VECCHIA C, et al. Role of reproductive factors on the risk of endometrial cancer. International journal of cancer 1998;76:784-6.
28. JORDAN SJ, NA R, JOHNATTY SE, et al. Breastfeeding and Endometrial Cancer Risk: An Analysis From the Epidemiology of Endometrial Cancer Consortium. Obstet Gynecol 2017;129:1059-67.
29. SCHMID D, BEHRENS G, KEIMLING M, JOCHEM C, RICCI C, LEITZMANN M. A systematic review and meta-analysis of physical activity and endometrial cancer risk. Eur J Epidemiol 2015;30:397-412.

30. MYUNG SK, JU W, CHOI HJ, KIM SC. Soy intake and risk of endocrine-related gynaecological cancer: a meta-analysis. Bjog 2009;116:1697-705.
31. AUDET-WALSH E, LEPINE J, GREGOIRE J, et al. Profiling of endogenous estrogens, their precursors, and metabolites in endometrial cancer patients: association with risk and relationship to clinical characteristics. The Journal of clinical endocrinology and metabolism 2011;96:E330-9.
32. ALLEN NE, KEY TJ, DOSSUS L, et al. Endogenous sex hormones and endometrial cancer risk in women in the European Prospective Investigation into Cancer and Nutrition (EPIC). Endocr Relat Cancer 2008;15:485-97.
33. MICHELS KA, BRINTON LA, WENTZENSEN N, et al. Postmenopausal Androgen Metabolism and Endometrial Cancer Risk in the Women's Health Initiative Observational Study. JNCI Cancer Spectrum 2019;3.
34. SOINI T, HURSKAINEN R, GRÉNMAN S, MÄENPÄÄ J, PAAVONEN J, PUKKALA E. Cancer risk in women using the levonorgestrel-releasing intrauterine system in Finland. Obstet Gynecol 2014;124:292-99.
35. JAREID M, THALABARD JC, AARFLOT M, BØVELSTAD HM, LUND E, BRAATEN T. Levonorgestrel-releasing intrauterine system use is associated with a decreased risk of ovarian and endometrial cancer, without increased risk of breast cancer. Results from the NOWAC Study. Gynecologic oncology 2018;149:127-32.
36. JANDA M, ROBLEDO KP, GEBSKI V, et al. Complete pathological response following levonorgestrel intrauterine device in clinically stage 1 endometrial adenocarcinoma: Results of a randomized clinical trial. Gynecologic oncology 2021;161:143-51.
37. AMANT F, MOERMAN P, NEVEN P, TIMMERMAN D, VAN LIMBERGEN E, VERGOTE I. Endometrial cancer. Lancet (London, England) 2005;366:491-505.
38. POTISCHMAN N, HOOVER RN, BRINTON LA, et al. Case-control study of endogenous steroid hormones and endometrial cancer. Journal of the National Cancer Institute 1996;88:1127-35.
39. ONSTAD MA, SCHMANDT RE, LU KH. Addressing the Role of Obesity in Endometrial Cancer Risk, Prevention, and Treatment. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2016;34:4225-30.
40. RENEHAN AG, ZWAHLEN M, EGGER M. Adiposity and cancer risk: new mechanistic insights from epidemiology. Nature Reviews Cancer 2015;15:484-98.
41. LATHAM A, SRINIVASAN P, KEMEL Y, et al. Microsatellite Instability Is Associated With the Presence of Lynch Syndrome Pan-Cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2019;37:286-95.
42. HARALDSDOTTIR S, RAFNAR T, FRANKEL WL, et al. Comprehensive population-wide analysis of Lynch syndrome in Iceland reveals founder mutations in MSH6 and PMS2. Nature Communications 2017;8:14755.

43. HAMPEL H, FRANKEL W, PANESCU J, et al. Screening for Lynch Syndrome (Hereditary Nonpolyposis Colorectal Cancer) among Endometrial Cancer Patients. Cancer research 2006;66:7810.
44. LU KH, SCHORGE JO, RODABAUGH KJ, et al. Prospective Determination of Prevalence of Lynch Syndrome in Young Women With Endometrial Cancer. Journal of Clinical Oncology 2007;25:5158-64.
45. MØLLER P, SEPPÄLÄ T, BERNSTEIN I, et al. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. Gut 2017;66:464-72.
46. SROCZYNSKI G, GOGOLLARI A, CONRADS-FRANK A, et al. Cost-Effectiveness of Early Detection and Prevention Strategies for Endometrial Cancer-A Systematic Review. Cancers 2020;12.
47. SCHMELER KM, LYNCH HT, CHEN LM, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome. N Engl J Med 2006;354:261-9.
48. MORICE P, LEARY A, CREUTZBERG C, ABU-RUSTUM N, DARAI E. Endometrial cancer. Lancet (London, England) 2016;387:1094-108.
49. IATRAKIS G, DIAKAKIS I, KOUROUNIS G, et al. Postmenopausal uterine bleeding. Clin Exp Obstet Gynecol 1997;24:157.
50. CLARKE MA, LONG BJ, SHERMAN ME, et al. Risk assessment of endometrial cancer and endometrial intraepithelial neoplasia in women with abnormal bleeding and implications for clinical management algorithms. American journal of obstetrics and gynecology 2020;223:549.e1-49.e13.
51. COLOMBO N, PRETI E, LANDONI F, et al. Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology 2013;24:vi33-vi38.
52. SEGEV Y, DAIN-SAGI L, LAVIE O, SAGI S, GEMER O. Is There a Survival Advantage in Diagnosing Endometrial Cancer in Asymptomatic Patients? A Systemic Review and Meta-analysis. J Obstet Gynaecol Can 2020;42:481-87.e2.
53. NARICE BF, DELANEY B, DICKSON JM. Endometrial sampling in low-risk patients with abnormal uterine bleeding: a systematic review and meta-synthesis. BMC Fam Pract 2018;19:135-35.
54. DIJKHUIZEN FP, MOL BW, BRÖLMANN HA, HEINTZ AP. The accuracy of endometrial sampling in the diagnosis of patients with endometrial carcinoma and hyperplasia: a meta-analysis. Cancer 2000;89:1765-72.
55. VAN HANEGEM N, BREIJER MC, SLOCKERS SA, et al. Diagnostic workup for postmenopausal bleeding: a randomised controlled trial. Bjog 2017;124:231-40.
56. CHANG YN, ZHANG Y, WANG YJ, WANG LP, DUAN H. Effect of hysteroscopy on the peritoneal dissemination of endometrial cancer cells: a meta-analysis. Fertility and sterility 2011;96:957-61.
57. CICINELLI E, TINELLI R, COLAFIGLIO G, et al. Risk of long-term pelvic recurrences after fluid minihysteroscopy in women with endometrial carcinoma: a controlled randomized study. Menopause 2010;17:511-5.

58. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. *WHO Classification of tumours. Female genital organ tumours.* Number of pages.
59. SOSLOW RA, TORNOS C, PARK KJ, et al. Endometrial Carcinoma Diagnosis: Use of FIGO Grading and Genomic Subcategories in Clinical Practice: Recommendations of the International Society of Gynecological Pathologists. International journal of gynecological pathology : official journal of the International Society of Gynecological Pathologists 2019;38 Suppl 1:S64-s74.
60. GILKS CB, OLIVA E, SOSLOW RA. Poor interobserver reproducibility in the diagnosis of high-grade endometrial carcinoma. Am J Surg Pathol 2013;37:874-81.
61. HAN G, SIDHU D, DUGGAN MA, et al. Reproducibility of histological cell type in high-grade endometrial carcinoma. Modern Pathology 2013;26:1594-604.
62. KÖBEL M, KALLOGER SE, BAKER PM, et al. Diagnosis of Ovarian Carcinoma Cell Type is Highly Reproducible: A Transcanadian Study. The American Journal of Surgical Pathology 2010;34.
63. HOANG LN, MCCONECHY MK, KÖBEL M, et al. Histotype-Genotype Correlation in 36 High-grade Endometrial Carcinomas. The American Journal of Surgical Pathology 2013;37.
64. VISSER NCM, REIJNEN C, MASSUGER L, NAGTEGAAL ID, BULTEN J, PIJNENBORG JMA. Accuracy of Endometrial Sampling in Endometrial Carcinoma: A Systematic Review and Meta-analysis. Obstet Gynecol 2017;130:803-13.
65. PECORELLI S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. International Journal of Gynecology & Obstetrics 2009;105:103-04.
66. CONCIN N, MATIAS-GUIU X, VERGOTE I, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society 2021;31:12-39.
67. ERIKSSON LS, LINDQVIST PG, FLÖTER RÅDESTAD A, et al. Transvaginal ultrasound assessment of myometrial and cervical stromal invasion in women with endometrial cancer: interobserver reproducibility among ultrasound experts and gynecologists. Ultrasound Obstet Gynecol 2015;45:476-82.
68. BOLLINENI VR, YTRE-HAUGE S, BOLLINENI-BALABAY O, SALVESEN HB, HALDORSEN IS. High Diagnostic Value of 18F-FDG PET/CT in Endometrial Cancer: Systematic Review and Meta-Analysis of the Literature. J Nucl Med 2016;57:879-85.
69. FASMER KE, GULATI A, DYBVIK JA, et al. Preoperative 18F-FDG PET/CT tumor markers outperform MRI-based markers for the prediction of lymph node metastases in primary endometrial cancer. Eur Radiol 2020.
70. LIU J, HAN L, JIAO Z. The diagnostic value of human epididymis protein 4 for endometrial cancer is moderate. Sci Rep 2021;11:575.
71. BRENNAN DJ, HACKETHAL A, METCALF AM, et al. Serum HE4 as a prognostic marker in endometrial cancer--a population based study. Gynecologic oncology 2014;132:159-65.

72. REIJNEN C, VISSER NC, KASIUS JC, et al. Improved preoperative risk stratification with CA-125 in low-grade endometrial cancer: a multicenter prospective cohort study. J Gynecol Oncol 2019;30:e70.
73. DI CELLO A, DI SANZO M, PERRONE FM, et al. DJ-1 is a reliable serum biomarker for discriminating high-risk endometrial cancer. Tumour Biol 2017;39:1010428317705746.
74. ENGERUD H, HOPE K, BERG HF, et al. Plasma growth differentiation factor-15 is an independent marker for aggressive disease in endometrial cancer. PLoS One 2019;14:e0210585.
75. REIJNEN C, GOGOU E, VISSER NCM, et al. Preoperative risk stratification in endometrial cancer (ENDORISK) by a Bayesian network model: A development and validation study. PLoS Med 2020;17:e1003111.
76. KANG S, KANG WD, CHUNG HH, et al. Preoperative Identification of a Low-Risk Group for Lymph Node Metastasis in Endometrial Cancer: A Korean Gynecologic Oncology Group Study. Journal of Clinical Oncology 2012;30:1329-34.
77. ABU-RUSTUM N, YASHAR CM, BRADLEY K, et al. Uterine Neoplasms, Version 1.2021, NCCN Clinical Practice Guidelines in Oncology. www.nccn.org, 2020.
78. THE NORWEGIAN DIRECTORATE OF HEALTH. Nasjonalt handlingsprogram med retningslinjer for gynekologisk kreft. Oslo, 2020.
79. SIGNORELLI M, LISSONI AA, CORMIO G, et al. Modified radical hysterectomy versus extrafascial hysterectomy in the treatment of stage I endometrial cancer: results from the ILIADE randomized study. Ann Surg Oncol 2009;16:3431-41.
80. LYU T, GUO L, CHEN X, et al. Ovarian preservation for premenopausal women with early-stage endometrial cancer: a Chinese retrospective study. J Int Med Res 2019;47:2492-98.
81. LAU H-Y, CHEN M-Y, KE Y-M, et al. Outcome of ovarian preservation during surgical treatment for endometrial cancer: A Taiwanese Gynecologic Oncology Group study. Taiwanese Journal of Obstetrics and Gynecology 2015;54:532-36.
82. BENEDETTI PANICI P, BASILE S, MANESCHI F, et al. Systematic pelvic lymphadenectomy vs. no lymphadenectomy in early-stage endometrial carcinoma: randomized clinical trial. Journal of the National Cancer Institute 2008;100:1707-16.
83. KITCHENER H, SWART AM, QIAN Q, AMOS C, PARMAR MK. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study. Lancet (London, England) 2009;373:125-36.
84. NAUMANN RW. The role of lymphadenectomy in endometrial cancer: Was the ASTEC trial doomed by design and are we destined to repeat that mistake? Gynecologic oncology 2012;126:5-11.
85. CRAGUN JM, HAVRILESKY LJ, CALINGAERT B, et al. Retrospective analysis of selective lymphadenectomy in apparent early-stage endometrial cancer. J Clin Oncol 2005;23:3668-75.

86. CHAN JK, CHEUNG MK, HUH WK, et al. Therapeutic role of lymph node resection in endometrioid corpus cancer: a study of 12,333 patients. Cancer 2006;107:1823-30.
87. TODO Y, KATO H, KANEUCHI M, WATARI H, TAKEDA M, SAKURAGI N. Survival effect of para-aortic lymphadenectomy in endometrial cancer (SEPAL study): a retrospective cohort analysis. Lancet (London, England) 2010;375:1165-72.
88. LEITAO MM, JR., ZHOU QC, GOMEZ-HIDALGO NR, et al. Patient-reported outcomes after surgery for endometrial carcinoma: Prevalence of lower-extremity lymphedema after sentinel lymph node mapping versus lymphadenectomy. Gynecologic oncology 2020;156:147-53.
89. HELGERS RJA, WINKENS B, SLANGEN BFM, WERNER HMJ. Lymphedema and Post-Operative Complications after Sentinel Lymph Node Biopsy versus Lymphadenectomy in Endometrial Carcinomas-A Systematic Review and Meta-Analysis. J Clin Med 2020;10.
90. CASARIN J, MULTINU F, ABU-RUSTUM N, et al. Factors influencing the adoption of the sentinel lymph node technique for endometrial cancer staging: an international survey of gynecologic oncologists. International Journal of Gynecologic Cancer 2019;29:60.
91. MOLONEY K, JANDA M, FRUMOVITZ M, et al. Development of a surgical competency assessment tool for sentinel lymph node dissection by minimally invasive surgery for endometrial cancer. International Journal of Gynecologic Cancer 2021;31:647.
92. BARLIN JN, KHOURY-COLLADO F, KIM CH, et al. The importance of applying a sentinel lymph node mapping algorithm in endometrial cancer staging: beyond removal of blue nodes. Gynecologic oncology 2012;125:531-5.
93. ZAHL ERIKSSON AG, DUCIE J, ALI N, et al. Comparison of a sentinel lymph node and a selective lymphadenectomy algorithm in patients with endometrioid endometrial carcinoma and limited myometrial invasion. Gynecologic oncology 2016;140:394-99.
94. SUIDAN RS, SUN CC, CANTOR SB, et al. Three Lymphadenectomy Strategies in Low-Risk Endometrial Carcinoma: A Cost-Effectiveness Analysis. Obstet Gynecol 2018;132:52-58.
95. KABAN A, TOPUZ S, ERDEM B, SOZEN H, NUMANOĞLU C, SALIHOĞLU Y. Is Omentectomy Necessary for Non-Endometrioid Endometrial Cancer. Gynecologic and Obstetric Investigation 2018;83:482-86.
96. JOO WD, SCHWARTZ PE, RUTHERFORD TJ, et al. Microscopic Omental Metastasis in Clinical Stage I Endometrial Cancer: A Meta-analysis. Annals of Surgical Oncology 2015;22:3695-700.
97. MARIANI A, WEBB MJ, KEENEY GL, ALETTI G, PODRATZ KC. Assessment of prognostic factors in stage IIIA endometrial cancer. Gynecologic oncology 2002;86:38-44.
98. RANDALL ME, FILIACI VL, MUSS H, et al. Randomized Phase III Trial of Whole-Abdominal Irradiation Versus Doxorubicin and Cisplatin Chemotherapy in Advanced Endometrial Carcinoma: A Gynecologic Oncology Group Study. Journal of Clinical Oncology 2006;24:36-44.

99. MAGGI R, LISSONI A, SPINA F, et al. Adjuvant chemotherapy vs radiotherapy in high-risk endometrial carcinoma: results of a randomised trial. Br J Cancer 2006;95:266-71.
100. SUSUMU N, SAGAE S, UDAGAWA Y, et al. Randomized phase III trial of pelvic radiotherapy versus cisplatin-based combined chemotherapy in patients with intermediate- and high-risk endometrial cancer: a Japanese Gynecologic Oncology Group study. Gynecologic oncology 2008;108:226-33.
101. MATEI D, FILIACI V, RANDALL ME, et al. Adjuvant Chemotherapy plus Radiation for Locally Advanced Endometrial Cancer. N Engl J Med 2019;380:2317-26.
102. DE BOER SM, POWELL ME, MILESHKIN L, et al. Adjuvant chemoradiotherapy versus radiotherapy alone in women with high-risk endometrial cancer (PORTEC-3): patterns of recurrence and post-hoc survival analysis of a randomised phase 3 trial. The Lancet Oncology 2019;20:1273-85.
103. WORTMAN BG, CREUTZBERG CL, PUTTER H, et al. Ten-year results of the PORTEC-2 trial for high-intermediate risk endometrial carcinoma: improving patient selection for adjuvant therapy. Br J Cancer 2018;119:1067-74.
104. KEYS HM, ROBERTS JA, BRUNETTO VL, et al. A phase III trial of surgery with or without adjunctive external pelvic radiation therapy in intermediate risk endometrial adenocarcinoma: a Gynecologic Oncology Group study. Gynecologic oncology 2004;92:744-51.
105. CREUTZBERG CL, VAN PUTTEN WLJ, KOPER PCM, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. The Lancet 2000;355:1404-11.
106. VAN DEN HEERIK ASVM, HOREWEG N, NOUT RA, et al. PORTEC-4a: international randomized trial of molecular profile-based adjuvant treatment for women with high-intermediate risk endometrial cancer. International Journal of Gynecologic Cancer 2020:ijgc-2020-001929.
107. MILLER DS, FILIACI VL, MANNEL RS, et al. Carboplatin and Paclitaxel for Advanced Endometrial Cancer: Final Overall Survival and Adverse Event Analysis of a Phase III Trial (NRG Oncology/GOG0209). J Clin Oncol 2020;38:3841-50.
108. ETHIER J-L, DESAUTELS DN, AMIR E, MACKAY H. Is hormonal therapy effective in advanced endometrial cancer? A systematic review and meta-analysis. Gynecologic oncology 2017;147:158-66.
109. VAN WEELDEN WJ, MASSUGER LFAG, E, PIJNENBORG JMA, ROMANO A. Anti-estrogen Treatment in Endometrial Cancer: A Systematic Review. Frontiers in Oncology 2019;9.
110. RUBINSTEIN M, HALPENNY D, MAKKER V, GRISHAM RN, AGHAJANIAN C, CADOO K. Retreatment with carboplatin and paclitaxel for recurrent endometrial cancer: A retrospective study of the Memorial Sloan Kettering Cancer Center experience. Gynecol Oncol Rep 2019;28:120-23.
111. HANAHAN D, WEINBERG ROBERT A. Hallmarks of Cancer: The Next Generation. Cell 2011;144:646-74.
112. HANAHAN D, WEINBERG RA. The Hallmarks of Cancer. Cell 2000;100:57-70.

113. ALEXANDROV LB, KIM J, HARADHVALA NJ, et al. The repertoire of mutational signatures in human cancer. Nature 2020;578:94-101.
114. CAMPBELL PJ, GETZ G, KORBEL JO, et al. Pan-cancer analysis of whole genomes. Nature 2020;578:82-93.
115. BELL DW, ELLENSON LH. Molecular Genetics of Endometrial Carcinoma. Annu Rev Pathol 2019;14:339-67.
116. URICK ME, BELL DW. Clinical actionability of molecular targets in endometrial cancer. Nat Rev Cancer 2019;19:510-21.
117. HAYES MP, WANG H, ESPINAL-WITTER R, et al. PIK3CA and PTEN mutations in uterine endometrioid carcinoma and complex atypical hyperplasia. Clinical cancer research : an official journal of the American Association for Cancer Research 2006;12:5932-5.
118. BERG A, HOIVIK EA, MJØS S, et al. Molecular profiling of endometrial carcinoma precursor, primary and metastatic lesions suggests different targets for treatment in obese compared to non-obese patients. Oncotarget 2015;6:1327-39.
119. URICK ME, BELL DW. Clinical actionability of molecular targets in endometrial cancer. Nature reviews Cancer 2019;19:510-21.
120. LEVINE DA, GETZ G, GABRIEL SB, et al. Integrated genomic characterization of endometrial carcinoma. Nature 2013;497:67-73.
121. MARABELLE A, LE DT, ASCIERTO PA, et al. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. J Clin Oncol 2020;38:1-10.
122. RODRIGUEZ AC, BLANCHARD Z, MAURER KA, GERTZ J. Estrogen Signaling in Endometrial Cancer: a Key Oncogenic Pathway with Several Open Questions. Hormones & cancer 2019;10:51-63.
123. HERNÁNDEZ-SILVA CD, VILLEGAS-PINEDA JC, PEREIRA-SUÁREZ AL. Expression and Role of the G Protein-Coupled Estrogen Receptor (GPR30/GPER) in the Development and Immune Response in Female Reproductive Cancers. Front Endocrinol (Lausanne) 2020;11.
124. KIM JJ, CHAPMAN-DAVIS E. Role of progesterone in endometrial cancer. Seminars in reproductive medicine 2010;28:81-90.
125. TROVIK J, WIK E, WERNER HM, et al. Hormone receptor loss in endometrial carcinoma curettage predicts lymph node metastasis and poor outcome in prospective multicentre trial. European journal of cancer (Oxford, England : 1990) 2013;49:3431-41.
126. AUSTIN H, AUSTIN JM, PARTRIDGE EE, HATCH KD, SHINGLETON HM. Endometrial Cancer, Obesity, and Body Fat Distribution. Cancer research 1991;51:568.
127. TANGEN IL, FASMER KE, KONINGS GF, et al. Blood steroids are associated with prognosis and fat distribution in endometrial cancer. Gynecologic oncology 2019;152:46-52.
128. JANZEN DM, ROSALES MA, PAIK DY, et al. Progesterone receptor signaling in the microenvironment of endometrial cancer influences its response to hormonal therapy. Cancer research 2013;73:4697-710.

129. NEAL AS, NUNEZ M, LAI T, et al. Expression of Stromal Progesterone Receptor and Differential Methylation Patterns in the Endometrium May Correlate with Response to Progesterone Therapy in Endometrial Complex Atypical Hyperplasia. Reproductive Sciences 2020;27:1778-90.
130. HÄGGSTRÖM M, RICHFIELD D. Diagram of the pathways of human steroidogenesis: Wikiversity, 2014 (vol 2021).
131. CORNEL KM, KRAKSTAD C, DELVOUX B, et al. High mRNA levels of 17β-hydroxysteroid dehydrogenase type 1 correlate with poor prognosis in endometrial cancer. Molecular and cellular endocrinology 2017;442:51-57.
132. SINREIH M, KNIFIC T, ANKO M, et al. The Significance of the Sulfatase Pathway for Local Estrogen Formation in Endometrial Cancer. Frontiers in pharmacology 2017;8:368-68.
133. COLLINS FS, VARMUS H. A new initiative on precision medicine. N Engl J Med 2015;372:793-5.
134. HENRY NL, HAYES DF. Cancer biomarkers. Mol Oncol 2012;6:140-46.
135. BALLMAN KV. Biomarker: Predictive or Prognostic? Journal of Clinical Oncology 2015;33:3968-71.
136. PARK J-Y, LEE JJ, CHOI HJ, et al. The Value of Preoperative Positron Emission Tomography/Computed Tomography in Node-Negative Endometrial Cancer on Magnetic Resonance Imaging. Annals of Surgical Oncology 2017;24:2303-10.
137. STÅLBERG K, BJURBERG M, BORGFELDT C, et al. Lymphovascular space invasion as a predictive factor for lymph node metastases and survival in endometrioid endometrial cancer – a Swedish Gynecologic Cancer Group (SweGCG) study. Acta Oncologica 2019;58:1628-33.
138. TANGEN IL, KOPPERUD RK, VISSER NC, et al. Expression of L1CAM in curettage or high L1CAM level in preoperative blood samples predicts lymph node metastases and poor outcome in endometrial cancer patients. Br J Cancer 2017;117:840-47.
139. MARIANI A, SEBO TJ, KATZMANN JA, et al. Endometrial cancer: can nodal status be predicted with curettage? Gynecologic oncology 2005;96:594-600.
140. HOANG LN, KINLOCH MA, LEO JM, et al. Interobserver Agreement in Endometrial Carcinoma Histotype Diagnosis Varies Depending on The Cancer Genome Atlas (TCGA)-based Molecular Subgroup. The American Journal of Surgical Pathology 2017;41.
141. KOMMOSS S, MCCONECHY MK, KOMMOSS F, et al. Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. Annals of oncology : official journal of the European Society for Medical Oncology 2018;29:1180-88.
142. MARCUS L, LEMERY SJ, KEEGAN P, PAZDUR R. FDA Approval Summary: Pembrolizumab for the Treatment of Microsatellite Instability-High Solid Tumors. Clinical cancer research : an official journal of the American Association for Cancer Research 2019;25:3753-58.
143. MAKKER V, TAYLOR MH, AGHAJANIAN C, et al. Lenvatinib Plus Pembrolizumab in Patients With Advanced Endometrial Cancer. Journal of Clinical Oncology 2020;38:2981-92.

144. ARORA S, BALASUBRAMANIAM S, ZHANG W, et al. FDA Approval Summary: Pembrolizumab plus Lenvatinib for Endometrial Carcinoma, a Collaborative International Review under Project Orbis. Clinical cancer research : an official journal of the American Association for Cancer Research 2020;26:5062-67.
145. FADER AN, ROQUE DM, SIEGEL E, et al. Randomized Phase II Trial of Carboplatin-Paclitaxel Compared with Carboplatin-Paclitaxel-Trastuzumab in Advanced (Stage III-IV) or Recurrent Uterine Serous Carcinomas that Overexpress Her2/Neu (NCT01367002): Updated Overall Survival Analysis. Clinical cancer research : an official journal of the American Association for Cancer Research 2020;26:3928-35.
146. MCALPINE JN, CHIU DS, NOUT RA, et al. Evaluation of treatment effects in patients with endometrial cancer and POLE mutations: An individual patient data meta-analysis. Cancer 2021;n/a.
147. MUSACCHIO L, CARUSO G, PISANO C, et al. PARP Inhibitors in Endometrial Cancer: Current Status and Perspectives. Cancer Manag Res 2020;12:6123-35.
148. JEPPESEN MM, MOGENSEN O, HANSEN DG, IACHINA M, KORSHOLM M, JENSEN PT. Detection of recurrence in early stage endometrial cancer - the role of symptoms and routine follow-up. Acta oncologica (Stockholm, Sweden) 2017;56:262-69.
149. HELSEDIREKTORATET. Nasjonalt handlingsprogram med retningslinjer for diagnostikk, behandling og oppfølging av gynekologisk kreft Oslo, 2016 (vol 2021).
150. JOLY F, MCALPINE J, NOUT R, ÅVALL-LUNDQVIST E, SHASH E, FRIEDLANDER M. Quality of Life and Patient-Reported Outcomes in Endometrial Cancer Clinical Trials: A Call for Action! International Journal of Gynecologic Cancer 2014;24:1693.
151. JANDA M, GEBSKI V, FORDER P, JACKSON D, WILLIAMS G, OBERMAIR A. Total laparoscopic versus open surgery for stage 1 endometrial cancer: the LACE randomized controlled trial. Contemp Clin Trials 2006;27:353-63.
152. WALKER JL, PIEDMONTE MR, SPIRTOS NM, et al. Laparoscopy Compared With Laparotomy for Comprehensive Surgical Staging of Uterine Cancer: Gynecologic Oncology Group Study LAP2. Journal of Clinical Oncology 2009;27:5331-36.
153. ACHOURI A, HUCHON C, BATS AS, BENSAID C, NOS C, LÉCURU F. Complications of lymphadenectomy for gynecologic cancer. Eur J Surg Oncol 2013;39:81-6.
154. VAN DE POLL-FRANSE LV, PIJNENBORG JM, BOLL D, et al. Health related quality of life and symptoms after pelvic lymphadenectomy or radiotherapy vs. no adjuvant regional treatment in early-stage endometrial carcinoma: a large population-based study. Gynecologic oncology 2012;127:153-60.
155. WEDIN M, STÅLBERG K, MARCICKIEWICZ J, et al. Incidence of lymphedema in the lower limbs and lymphocyst formation within one year of surgery for endometrial cancer: A prospective longitudinal multicenter study. Gynecologic oncology 2020;159:201-08.
156. VOLPI L, SOZZI G, CAPOZZI VA, et al. Long term complications following pelvic and para-aortic lymphadenectomy for endometrial cancer, incidence and

potential risk factors: a single institution experience. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society 2019;29:312-19.
157. YOST KJ, CHEVILLE AL, AL-HILLI MM, et al. Lymphedema after surgery for endometrial cancer: prevalence, risk factors, and quality of life. Obstet Gynecol 2014;124:307-15.
158. SALEHI S, BRANDBERG Y, AVALL-LUNDQVIST E, et al. Long-term quality of life after comprehensive surgical staging of high-risk endometrial cancer - results from the RASHEC trial. Acta oncologica (Stockholm, Sweden) 2018:1-6.
159. LINDQVIST E, WEDIN M, FREDRIKSON M, KJOLHEDE P. Lymphedema after treatment for endometrial cancer - A review of prevalence and risk factors. European journal of obstetrics, gynecology, and reproductive biology 2017;211:112-21.
160. DE BOER SM, NOUT RA, JÜRGENLIEMK-SCHULZ IM, et al. Long-Term Impact of Endometrial Cancer Diagnosis and Treatment on Health-Related Quality of Life and Cancer Survivorship: Results From the Randomized PORTEC-2 Trial. Int J Radiat Oncol Biol Phys 2015;93:797-809.
161. NOUT RA, PUTTER H, JÜRGENLIEMK-SCHULZ IM, et al. Five-year quality of life of endometrial cancer patients treated in the randomised Post Operative Radiation Therapy in Endometrial Cancer (PORTEC-2) trial and comparison with norm data. European journal of cancer (Oxford, England : 1990) 2012;48:1638-48.
162. NOUT RA, VAN DE POLL-FRANSE LV, LYBEERT ML, et al. Long-term outcome and quality of life of patients with endometrial carcinoma treated with or without pelvic radiotherapy in the post operative radiation therapy in endometrial carcinoma 1 (PORTEC-1) trial. J Clin Oncol 2011;29:1692-700.
163. DE BOER SM, POWELL ME, MILESHKIN L, et al. Toxicity and quality of life after adjuvant chemoradiotherapy versus radiotherapy alone for women with high-risk endometrial cancer (PORTEC-3): an open-label, multicentre, randomised, phase 3 trial. The Lancet Oncology 2016;17:1114-26.
164. JOLY F, MCALPINE J, NOUT R, AVALL-LUNDQVIST E, SHASH E, FRIEDLANDER M. Quality of life and patient-reported outcomes in endometrial cancer clinical trials: a call for action! International journal of gynecological cancer : official journal of the International Gynecological Cancer Society 2014;24:1693-9.
165. CARTER J, HUANG HQ, ARMER J, et al. GOG 244 - The LymphEdema and Gynecologic cancer (LEG) study: The association between the gynecologic cancer lymphedema questionnaire (GCLQ) and lymphedema of the lower extremity (LLE). Gynecologic oncology 2019;155:452-60.
166. YOST KJ, CHEVILLE AL, WEAVER AL, AL HILLI M, DOWDY SC. Development and Validation of a Self-Report Lower-Extremity Lymphedema Screening Questionnaire in Women. Physical Therapy 2013;93:694-703.
167. METHLIE P, HUSTAD SS, KELLMANN R, et al. Multisteroid LC-MS/MS assay for glucocorticoids and androgens, and its application in Addison's disease. Endocrine connections 2013;2:125-36.

168. BERTELSEN BE, KELLMANN R, VISTE K, et al. An Ultrasensitive Routine LC-MS/MS Method for Estradiol and Estrone in the Clinically Relevant Sub-Picomolar Range. J Endocr Soc 2020;4:bvaa047.
169. TANGEN IL, ONYANGO TB, KOPPERUD R, et al. Androgen receptor as potential therapeutic target in metastatic endometrial cancer. Oncotarget 2016;7:49289-98.
170. TANGEN IL, VENERIS JT, HALLE MK, et al. Expression of glucocorticoid receptor is associated with aggressive primary endometrial cancer and increases from primary to metastatic lesions. Gynecologic oncology 2017;147:672-77.
171. WIK E, RAEDER MB, KRAKSTAD C, et al. Lack of estrogen receptor-alpha is associated with epithelial-mesenchymal transition and PI3K alterations in endometrial carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research 2013;19:1094-105.
172. TANGEN IL, WERNER HM, BERG A, et al. Loss of progesterone receptor links to high proliferation and increases from primary to metastatic endometrial cancer lesions. European journal of cancer (Oxford, England : 1990) 2014;50:3003-10.
173. SUBRAMANIAN A, TAMAYO P, MOOTHA VK, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proceedings of the National Academy of Sciences 2005;102:15545.
174. MAULAND KK, ENG O, YTRE-HAUGE S, et al. High visceral fat percentage is associated with poor outcome in endometrial cancer. Oncotarget 2017;8:105184-95.
175. KINKEL K, FORSTNER R, DANZA FM, et al. Staging of endometrial cancer with MRI: guidelines of the European Society of Urogenital Imaging. Eur Radiol 2009;19:1565-74.
176. ZANDBERGEN N, DE ROOIJ BH, VOS MC, et al. Changes in health-related quality of life among gynecologic cancer survivors during the two years after initial treatment: a longitudinal analysis. Acta oncologica (Stockholm, Sweden) 2019;58:790-800.
177. FERRANDINA G, PETRILLO M, MANTEGNA G, et al. Evaluation of quality of life and emotional distress in endometrial cancer patients: A 2-year prospective, longitudinal study. Gynecologic oncology 2014;133:518-25.
178. SMITS A, LOPES A, BEKKERS R, GALAAL K. Body mass index and the quality of life of endometrial cancer survivors--a systematic review and meta-analysis. Gynecologic oncology 2015;137:180-7.
179. FAYERS PM AN, BJORDAL K, GROENVOLD M, CURRAN D, BOTTOMLEY A. The EORTC QLQ-C30 Scoring Manual (3rd Edition). Brussels: European Organisation for Research and Treatment of Cancer, 2001.
180. NICHOL AD, BAILEY M, COOPER DJ. Challenging issues in randomised controlled trials. Injury 2010;41 Suppl 1:S20-3.
181. BEDARD PL, KRZYZANOWSKA MK, PINTILIE M, TANNOCK IF. Statistical power of negative randomized controlled trials presented at American Society for Clinical Oncology annual meetings. J Clin Oncol 2007;25:3482-7.

182. ROTHWELL PM. External validity of randomised controlled trials: "to whom do the results of this trial apply?". Lancet (London, England) 2005;365:82-93.
183. ANGLEMYER A, HORVATH HT, BERO L. Healthcare outcomes assessed with observational study designs compared with those assessed in randomized trials. Cochrane Database Syst Rev 2014:Mr000034.
184. CARLSON MDA, MORRISON RS. Study design, precision, and validity in observational studies. J Palliat Med 2009;12:77-82.
185. GREBE SK, SINGH RJ. LC-MS/MS in the Clinical Laboratory - Where to From Here? The Clinical biochemist Reviews 2011;32:5-31.
186. BOLELLI G, MUTI P, MICHELI A, et al. Validity for epidemiological studies of long-term cryoconservation of steroid and protein hormones in serum and plasma. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 1995;4:509-13.
187. HOLL K, LUNDIN E, KAASILA M, et al. Effect of long-term storage on hormone measurements in samples from pregnant women: the experience of the Finnish Maternity Cohort. Acta oncologica (Stockholm, Sweden) 2008;47:406-12.
188. VOGEL C, MARCOTTE EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet 2012;13:227-32.
189. KOUSSOUNADIS A, LANGDON SP, UM IH, HARRISON DJ, SMITH VA. Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. Scientific Reports 2015;5:10775.
190. COENS C, PE M, DUECK AC, et al. International standards for the analysis of quality-of-life and patient-reported outcome endpoints in cancer randomised controlled trials: recommendations of the SISAQOL Consortium. The Lancet Oncology 2020;21:e83-e96.
191. LUCKETT T, KING MT, BUTOW PN, et al. Choosing between the EORTC QLQ-C30 and FACT-G for measuring health-related quality of life in cancer clinical research: issues, evidence and recommendations. Annals of Oncology 2011;22:2179-90.
192. FREDHEIM OMS, BORCHGREVINK PC, SALTNES T, KAASA S. Validation and Comparison of the Health-Related Quality-of-Life Instruments EORTC QLQ-C30 and SF-36 in Assessment of Patients with Chronic Nonmalignant Pain. Journal of Pain and Symptom Management 2007;34:657-65.
193. GIESINGER JM, LOTH FLC, AARONSON NK, et al. Thresholds for clinical importance were established to improve interpretation of the EORTC QLQ-C30 in clinical practice and research. Journal of Clinical Epidemiology 2020;118:1-8.
194. COCKS K, KING MT, VELIKOVA G, et al. Evidence-based guidelines for interpreting change scores for the European Organisation for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30. European journal of cancer (Oxford, England : 1990) 2012;48:1713-21.
195. OSOBA D, RODRIGUES G, MYLES J, ZEE B, PATER J. Interpreting the significance of changes in health-related quality-of-life scores. J Clin Oncol 1998;16:139-44.

196. COHEN J. *Statistical power analysis for the behavioral sciences.* New York: Lawrence Erlbaum Associates; Number of pages.
197. NORMAN GR, SLOAN JA, WYRWICH KW. Interpretation of Changes in Health-related Quality of Life: The Remarkable Universality of Half a Standard Deviation. Medical Care 2003;41.
198. FIELD A, MILES J, FIELD Z. *Discovering statistics using R.* London, England: SAGE Publications; Number of pages.
199. TSAI W-H, WONG C-H, DAI S-H, TSAI C-H, ZENG Y-H. Adrenal Tumor Mimicking Non-Classic Congenital Adrenal Hyperplasia. Front Endocrinol (Lausanne) 2020;11:526287-87.
200. MILLER WL, AUCHUS RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev 2011;32:81-151.
201. BARNES RB, ROSENFIELD RL, BURSTEIN S, EHRMANN DA. Pituitary-ovarian responses to nafarelin testing in the polycystic ovary syndrome. N Engl J Med 1989;320:559-65.
202. KONINGS G, BRENTJENS L, DELVOUX B, et al. Intracrine Regulation of Estrogen and Other Sex Steroid Levels in Endometrium and Non-gynecological Tissues; Pathology, Physiology, and Drug Discovery. Front Pharmacol 2018;9:940.
203. JARZABEK K, KODA M, WALENTOWICZ-SADLECKA M, GRABIEC M, LAUDANSKI P, WOLCZYNSKI S. Altered expression of ERs, aromatase, and COX2 connected to estrogen action in type 1 endometrial cancer biology. Tumor Biology 2013;34:4007-16.
204. BLANCO JR LZ, KUHN E, MORRISON JC, BAHADIRLI-TALBOTT A, SMITH-SEHDEV A, KURMAN RJ. Steroid hormone synthesis by the ovarian stroma surrounding epithelial ovarian tumors: a potential mechanism in ovarian tumorigenesis. Modern Pathology 2017;30:563-76.
205. TODO Y, OKAMOTO K, HAYASHI M, et al. A validation study of a scoring system to estimate the risk of lymph node metastasis for patients with endometrial cancer for tailoring the indication of lymphadenectomy. Gynecologic oncology 2007;104:623-28.
206. SCHIAVONE MB, SCELZO C, STRAIGHT C, et al. Survival of Patients with Serous Uterine Carcinoma Undergoing Sentinel Lymph Node Mapping. Ann Surg Oncol 2017;24:1965-71.
207. SCHIAVONE MB, ZIVANOVIC O, ZHOU Q, et al. Survival of Patients with Uterine Carcinosarcoma Undergoing Sentinel Lymph Node Mapping. Ann Surg Oncol 2016;23:196-202.
208. ALLAM O, PARK KE, CHANDLER L, et al. The impact of radiation on lymphedema: a review of the literature. Gland Surg 2020;9:596-602.
209. SCOTT MG. When do new biomarkers make economic sense? Scandinavian Journal of Clinical and Laboratory Investigation 2010;70:90-95.
210. HAY CM, DONOVAN HS, CAMPBELL GB, TAYLOR SE, WANG L, COURTNEY-BROOKS M. Chemotherapy in older adult gynecologic oncology patients: Can a phenotypic frailty score predict tolerance? Gynecologic oncology 2019;152:304-09.

211. FERRERO A, VILLA M, TRIPODI E, FUSO L, MENATO G. Can Vulnerable Elders Survey-13 predict the impact of frailty on chemotherapy in elderly patients with gynaecological malignancies? Medicine (Baltimore) 2018;97:e12298.
212. SIA TY, WEN T, CHAM S, FRIEDMAN AM, WRIGHT JD. The effect of frailty on postoperative readmissions, morbidity, and mortality in endometrial cancer surgery. Gynecologic oncology 2021;161:353-60.
213. EVERETT E, TAMIMI H, GREER B, et al. The effect of body mass index on clinical/pathologic features, surgical morbidity, and outcome in patients with endometrial cancer. Gynecologic oncology 2003;90:150-7.

Gynecologic Oncology 156 (2020) 400–406

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Blood steroid levels predict survival in endometrial cancer and reflect tumor estrogen signaling

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HIGHLIGHTS

- A clinically available panel of steroid hormones was analyzed in 100 endometrial cancer patient plasma samples.
- Low 17OH-progesterone, 11-deoxycortisol and androstenedione associated to aggressive tumor characteristics.
- 17OH-progesterone and 11-deoxycortisol predicted poor survival independent of preoperative risk classification.
- Genes associated with estrogen signaling were enriched in tumors of patients with high steroid levels.

ARTICLE INFO

Article history:
Received 25 October 2019
Received in revised form 28 November 2019
Accepted 29 November 2019
Available online 6 December 2019

Keywords:
Endometrial cancer
Steroid hormones
Survival
Estrogen signaling
Circulating biomarkers
Fat distribution

ABSTRACT

Objective. Blood-based biomarkers are attractive due to ease of sampling and standardized measurement technology, reducing obstacles to clinical implementation. The objective of this study was to evaluate a clinically available method of steroid hormone measurement for its prognostic potential in endometrial cancer.

Methods. We quantified seven steroid hormones by liquid chromatography-tandem mass spectrometry in 100 endometrial cancer patients from a prospective cohort. Abdominal fat distribution was assessed from abdominal computed tomography (CT) scans. Steroid hormone levels were compared to clinical characteristics, fat distribution and gene expression in primary tumor samples.

Results. Low levels of 17OH-progesterone, 11-deoxycortisol and androstenedione were associated with aggressive tumor characteristics and poor disease specific survival ($p = .003$, $p = .001$ and $p = .02$ respectively). Adjusting for preoperative risk based on histological type and grade, low 17OH-progesterone and 11-deoxycortisol independently predicted poor outcome with hazard ratios of 2.69 ($p = .033$, 95%CI: 1.09–6.68) and 3.40 ($p = .020$, 1.21–9.51), respectively. Tumors from patients with low steroid level displayed increased expression of genes related to mitosis and cell cycle progression, whereas high steroid level was associated with up-regulated estrogen signaling and genes associated with inflammation. Estrone and estradiol correlated to abdominal fat volume in all compartments (total, visceral, subcutaneous, $p < .001$ for all), but not to the visceral fat proportion. Patients with higher levels of circulating estrogens had increased expression of estrogen signaling related genes.

Conclusion. Low levels of certain endogenous steroids are associated with aggressive tumor traits and poor survival and may provide preoperative information independent of histological biomarkers already in use.

1. Introduction

Endometrial cancer is the most common gynecologic malignancy in industrialized countries, and the incidence is rising [1,2]. Prognosis is generally good due to early detection and predominance of low-grade endometrioid histology, while high risk disease, comprising non-

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https://doi.org/10.1016/j.ygyno.2019.11.123
0090-8258/

endometrioid tumors and grade 3 endometrioid disease, carries markedly poorer prognosis [3]. However, due to the much higher incidence of endometrioid tumors, the absolute number of recurrences is significant also in this group [4,5]. Identification of biomarkers that can aid selection of patients for optimal surgical and adjuvant treatment independent of histological parameters is vital to improve outcome.

Several prognostic and diagnostic biomarkers have been identified in tumor biopsies [6] but few have so far been implemented in the clinic to improve treatment for endometrial cancer patients. In some institutions, hormone receptor status is assessed as a supplement to traditional histological evaluation, and the design and validation of combined molecular classifiers is ongoing, driven by initiatives like ProMisE and TransPORTEC [7,8]. Compared to these tissue-based biomarkers, blood-based biomarkers do not require a biopsy and thus represent less invasive clinical tools to predict prognosis and to plan patient treatment, and pose little technical challenge in implementation. Several blood biomarkers have already been investigated in endometrial cancer, e.g. Ca-125 is shown to have prognostic value and identifies advanced disease and lymph node metastasis [9]. Other blood based biomarkers such as HE4, GDF-15, and DJ-1 have also been found promising [10–12], but lack validation in a prospective implementation setting.

Although the influence of hormone receptor expression on prognosis has been extensively researched [13–15], few studies have evaluated the importance of endogenous steroid levels other than estrogen metabolites [16–18], and to some extent androstenedione (A4) and testosterone (T) [19–22]. These studies have mostly focused on risk of acquiring disease rather than biomarker properties. We have previously demonstrated differences in levels of several steroids in blood samples in a matched patient series of long vs short surviving endometrial cancer patients [23].

For clinical implementation of a blood-based test, easy and reliable methods for detection are vital. In this study, we measured levels of circulating steroids in endometrial cancer patients by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). We selected a panel of relevant steroids related to sex hormone synthesis, used clinically for diagnosing endocrinological disorders. This panel was supplemented with measurements of estrone (E1) and estradiol (E2) using a novel sensitive protocol, properly quantifying postmenopausal estrogen levels in plasma. We explored the relationship to body mass, fat distribution variables, associations to clinicopathologic characteristics of the disease and patient survival. Finally, we analyzed differences in gene expression to identify links between host steroid levels and tumor biology. The aim of the study was to evaluate the prognostic value of circulating steroid levels in endometrial cancer patients.

2. Methods

2.1. Ethical considerations

The study has been approved according to Norwegian legislation by the Western Regional Committee for medical and health Research Ethics (REK 2009/2315, REK 2014/1907, REK 2018/594, REK 2019/1020). All included patients gave written informed consent.

2.2. Patient series

A population based endometrial cancer patient series was prospectively collected from 2001 to 2015 in Hordaland County (Norway). Patients were surgically staged according to the International Federation of Gynecology and Obstetrics (FIGO) 2009 criteria. Clinical and pathological variables including age at diagnosis, FIGO stage, histological subtype, grade, and follow up data were collected by review of medical records as previously described [24]. From this series, 100 postmenopausal patients included from 2009 to 2013 were selected to reflect clinicopathological characteristics of the whole prospective cohort (Supplementary Table S1). Median follow-up was 67 months (range 1–116) and minimum 60 months for all survivors. During follow-up, 20 cancer specific deaths were registered, and six patients were censored due to non-cancer deaths.

Immunohistochemical staining and evaluation of hormone receptors has been performed previously for this cohort [14,25–27]. Expression data for estrogen receptor (ERα), progesterone receptor (PR), Androgen Receptor (AR) and Glucocorticoid Receptor (GR) was available for 93, 94, 87 and 76 patients, respectively.

2.3. Steroid analysis

EDTA-blood was obtained before primary surgery, and prior to administration of any anesthetic medications, from 100 patients with endometrial cancer. The blood samples were centrifuged at 1600*g* for 15 min and the plasma was stored at −80 °C. Median storage time before analysis was 66 months (range 47–104 months). Steroids were measured at the Hormone Laboratory, Haukeland University Hospital, Bergen Norway, using the routinely applied LC-MS/MS method for plasma analysis of 17-hydroxyprogesterone (17-OHP), 11-deoxycortisol (11-DOC), cortisol (CORT), androstenedione (A4) and testosterone (T) previously described [28]. Briefly, isotope-labeled internal standards were added to 85 μL plasma and processed by liquid-liquid extraction. The steroids were resolved by ultra-high-pressure chromatography on a reverse phase column, and detected by triple-quadrupole mass spectrometry. For analysis of estrone (E1) and estradiol (E2), a recently developed optimized protocol was used, with limits of quantification of 0.3 pmol/L and 0.6 pmol/L respectively, thus allowing quantification within the postmenopausal ranges of these hormones [29]. Both LC-MS/MS methods are accredited according to ISO 15189:2012.

17-OHP, CORT, A4 and T were measured for all 100 patients. For 11-DOC, 98 patient samples were measured, two samples were not analyzed due to technical difficulties. For six patients plasma level of 17-OHP was below the detectable threshold of 0.2 nmol/L. For one of these patients A4 and T were also below the threshold (0.2 nmol/L and 0.1 nmol/L respectively). These levels were set to the lowest detectable value for each steroid and included in non-parametric analyses. Plasma level of progesterone was measured, but was below measurement threshold for all patients (<0.5 nmol/L) and was subsequently excluded.

E1 and E2 plasma levels were obtained from 96 patients. No values were below analytic range, measurements above the analytic range (above the highest calibrator, $n = 7$ for E1 and $n = 3$ for E2) were analyzed as ranked values in non-parametric analysis.

2.4. Estimation of fat distribution from CT scans

Complete diagnostic abdominal contrast-enhanced Computer Tomography (CT) scans were available for 83 patients and evaluated for assessment of abdominal fat volumes as previously described [23]. The software iNtuition (TeraRecon Inc.; San Mateo, CA, USA), was used to analyze cross-sectional CT images from the upper right diaphragm to L5/S1-level, segmenting pixels with values for Hounsfield units (HU) corresponding to adipose tissue (−195 to −45 HU). If necessary, the correct segmentation between visceral and subcutaneous fat compartments was adjusted by the operator. Both the visceral abdominal fat volume (VAV; cm^3) and the subcutaneous abdominal fat volumes (SAV; cm^3) were estimated, and the sum of these was the total abdominal fat volume (TAV; cm^3). The percentage of visceral fat was calculated ([VAV/TAV] x 100; VAV%). In addition, waist circumference was measured in an axial image at the L3/L4 level.

2.5. Gene expression analysis

Gene expression data from tumor tissue was available for all included patients and has been published previously [25]. Briefly, RNA

was extracted from fresh frozen tissue using the RNeasy Mini Kit (Qiagen, Germany), hybridized to Agilent Whole Human Genome Microarray 44 k (Cat. No. G4112F), scanned and normalized as previously described. We limited mRNA expression analysis to the endometrioid subgroup ($n = 77$). Associations between blood steroid levels and activated signaling pathways in tumor tissue were investigated using J-express 2012 software (Molmine, Bergen). Differentially expressed genes were identified by running Significance Analysis of Microarrays (SAM) method. Gene Set Enrichment Analysis (GSEA) was performed in J-express with Hallmark gene-sets from MSigDB (Broad Institute, US) [30].

2.6. Statistical analysis

Statistical analyses were performed using the software package SPSS 25.0 (SPSS Inc., Chicago, IL). Probability of <0.05 was defined as statistically significant and all tests were two sided. Plasma concentrations were analyzed with non-parametric tests, i. e. Mann-Whitney *U* test for independent samples. Correlations were assessed by Spearman's rank correlation (ρ = rho). Cut-off for survival analysis grouping was set applying Receiver Operating Characteristic (ROC) analysis for disease specific death during follow-up. Area under curve (AUC) >0.6 was set as acceptable limit for further analysis and cut-off was determined using highest Youden Index (sensitivity + specificity-1) (Supplementary Fig. S1). Univariate survival analyses were performed using the Kaplan-Meier (product-limit) method. Entry date was the date of primary surgery, and time to death due to endometrial cancer was the endpoint (disease specific survival). Survival between groups was compared using the log-rank test (Mantel-Cox). Multivariate survival analysis was carried out by the Cox proportional hazards method, with single step enter.

3. Results

3.1. Low plasma levels of 17-OHP, 11-DOC and A4 associate with aggressive phenotype

Employing two clinically available panels of steroid hormone analyses, we measured levels of seven relevant steroid hormones and intermediates using LC-MS/MS (Fig. 1A). Because of the known marked diurnal variation of CORT, it was excluded from further analysis. The remaining six steroid hormones had distributions as shown in Fig. 1B. Analyzed steroids were generally significantly positively correlated to each other, ranging from moderate to strong (Spearman's rho: 0.34–0.92). 11-DOC however, did not correlate significantly to any of the estrogens. There was no correlation between storage time and level of any of the hormones (Supplementary Table S2). The distribution of hormone levels in our cohort was comparable to previously published data on LC-MS/MS reference intervals of steroids in healthy postmenopausal women [31] (Supplementary Fig. S2). Plasma levels of steroids were investigated for any associations with clinicopathological features of endometrial cancer (Table 1). Overall, lower median levels of steroid hormones associated with more aggressive endometrial cancers. Specifically, low 17-OHP was significantly associated with preoperative high-risk classification (grade 3 endometrioid or non-endometrioid histology, $p = .032$), post-operative non-endometrioid histology, ($p = .009$) and metastatic lymph nodes ($p = .046$) (Table 1). Low 11-DOC associated with advanced FIGO stage (III-IV, $p = .02$) and non-endometrioid histology ($p = .002$). Low A4 associated with non-endometrioid histology ($p = .023$). T did not associate with any of the investigated tumor characteristics. For E1 and E2, low levels were associated with high histologic grade (grade 3 compared to 1–2, p≤=0.015) in the surgical specimen.

When restricting the analyses to patients with endometrioid histology ($n = 77$), low 17-OHP was associated with advanced FIGO stage ($p = .014$), lymph node metastasis ($p = .029$) and deep myometrial infiltration ($p = .028$). Among the other hormones, low A4 associated with advanced FIGO stage (p = .028) and low T with lymph node metastases ($p = .025$) (Supplementary Table S3). No significant association was found for 11-DOC in the endometrioid endometrial cancer subgroup.

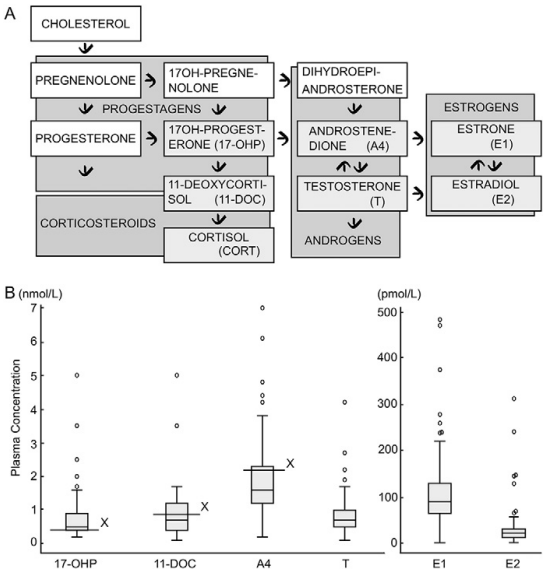


Fig. 1. A) Measured steroid hormones (in gray) and their positions and relations in the steroid synthesis. B) Distribution of plasma levels of the measured steroid hormones in the study cohort. Boxes represent median values with interquartile ranges, whiskers represent non-outlying max and min values (<1.5 times the interquartile range). The cutoff for survival analysis marked with x. One extreme outlier for E2 (1189 pmol/L) is not displayed.

3.2. Low levels of 17-OHP, 11-DOC and A4 predict poor survival in endometrial cancer

To analyze if any of the plasma steroids had prognostic value, optimal plasma level cut-offs were defined by ROC-curve analysis (Supplementary Fig. S1). Plasma levels of T, E1 or E2 did not predict disease specific death (not shown). In univariate analysis, low preoperative levels of 17-OHP, 11-DOC and A4 were associated with poor disease specific survival (Fig. 2). In separate cox regression models for 17-OHP, 11-DOC and A4, including the hormone and preoperative risk classification (based on histological type and grade), 17-OHP and 11-DOC were still independent prognostic factors with hazard ratios of 2.69 ($p = .033$, 95% CI: 1.09–6.68) and 3.40 ($p = .020$, 95% CI 1.21–9.51), respectively (Table 2). The adjusted hazard ratio of A4 did not reach statistical significance ($p = .08$).

3.3. High E1 and E2 levels are associated with overweight and high hormone receptor expression in primary tumors

E1 and E2 were positively correlated with BMI, waist circumference, TAV, SAV and VAV (ρ = 0.46–0.63) (Table 3). T had a weak positive correlation with BMI and subcutaneous abdominal fat volume (ρ = 0.23 and ρ = 0.22, $p < .05$ for both). None of the other steroids was associated with body fat variables.

We found plasma steroid levels to be higher in hormone receptor positive primary tumors (Supplementary Table S4). In PR positive tumors, patient plasma levels of E1 and E2 were significantly higher ($p = .003$ and $p < .001$ respectively) as well as levels of 17-OHP ($p =$

Table 1
Clinical-pathological characteristics related to median steroid hormone levels (10–90 percentile) in endometrial cancer patients. Statistically significant p-values (<.05) in bold.

	17OH-Progesterone		11-Deoxycortisol		Androstenedione		Testosterone		Estrone		Estradiol	
	n = 100		*n* = 98		n = 100		n = 100		*n* = 96		n = 96	
	nmol/L	p	nmol/L	p	nmol/L	p	nmol/L	p	pmol/L	p	pmol/L	p
Age		0.17		0.10		**0.001**		0.59		0.81		0.93
<66 (*n* = 41)	0.6 (0.2–1.9)		0.8 (0.3–1.7)		2.0 (1.0–4.1)		0.7 (0.4–1.6)		106 (43–217)		20 (5–128)	
≥66 (*n* = 59)	0.5 (0.2–1.0)		0.6 (0.3–1.4)		1.4 (0.9–2.6)		0.7 (0.3–1.2)		89 (47–220)		22 (8–51)	
Preoperative risk[a]		**0.032**		**0.048**		0.20		0.34		0.76		0.31
Low (*n* = 57)	0.6 (0.2–1.3)		0.7 (0.3–1.7)		1.7 (0.9–3.6)		0.8 (0.4–1.5)		106 (44–212)		22 (8–65)	
High (*n* = 43)	0.5 (0.2–1.0)		0.6 (0.3–1.3)		1.4 (0.9–3.1)		0.6 (0.3–1.5)		84 (44–238)		17 (5–54)	
FIGO-09 stage		0.10		**0.02**		0.06		0.11		0.65		0.68
I-II (*n* = 87)	0.6 (0.3–1.2)		0.7 (0.3–1.6)		1.6 (0.9–3.6)		0.7 (0.4–1.5)		89 (44–216)		20 (8–61)	
III-IV (*n* = 13)	0.4 (0.2–1.0)		0.6 (0.1–1.1)		1.3 (0.9–2.0)		0.5 (0.2–1.1)		108 (45–226)		22 (6–108)	
Histologic type[b]		**0.009**		**0.002**		**0.023**		0.08		0.41		0.21
EEC (n = 77)	0.6 (0.2–1.2)		0.8 (0.3–1.6)		1.6 (0.9–3.6)		0.8 (0.4–1.5)		106 (45–216)		22 (8–65)	
Serous (*n* = 14)	0.5 (0.2–1.4)		0.6 (0.1–1.0)		1.4 (0.8–3.0)		0.8 (0.4–1.2)		97 (45–236)		20 (7–98)	
Others (n = 9)[c]	0.4 (0.2–0.5)		0.4 (0.3–1.0)		1.2 (0.7–1.7)		0.5 (0.2–0.6)		65 (31–86)		10 (6–19)	
Histologic grade[d]		0.35		0.78		0.19		0.08		**0.012**		**0.015**
Grade 1/2 (*n* = 56)	0.6 (0.2–1.8)		0.8 (0.3–1.6)		1.7 (0.9–3.9)		0.8 (0.4–1.6)		108 (50–243)		24 (10–104)	
Grade 3 (*n* = 20)	0.6 (0.2–1.1)		0.9 (0.3–1.7)		1.6 (0.8–2.8)		0.7 (0.2–1.5)		72 (41–134)		15 (5–41)	
Lymph node status		**0.046**		0.15		0.14		0.08		0.62		0.49
Negative (*n* = 79)	0.5 (0.2–1.4)		0.7 (0.3–1.5)		1.6 (0.9–3.6)		0.7 (0.4–1.5)		89 (45–210)		20 (9–59)	
Positive (n = 8)	0.4 (0.2–0.5)		0.6 (0.2–0.8)		1.3 (1.0–1.6)		0.5 (0.3–0.6)		81 (50–108)		19 (6–23)	
Myometrial infiltr.		0.38		0.22		0.07		0.29		0.10		0.11
<50% (*n* = 53)	0.6 (0.2–1.1)		0.8 (0.3–1.7)		1.7 (1.0–3.8)		0.7 (0.4–1.6)		94 (54–257)		22 (10−122)	
≥50% (*n* = 47)	0.5 (0.2–1.2)		0.7 (0.3–1.3)		1.5 (0.9–3.3)		0.7 (0.3–1.4)		83 (36–203)		17 (6–52)	

Statistical comparisons are done with Mann-Whitney *U* test for independent samples. EEC: Endometrioid endometrial cancer.
[a] Based on preoperative biopsy/curettage; low if EEC grade 1–2, high if grade 3 or non-endometroid histology.
[b] *P*-values for Endometroid vs. All non-endometroid histologies.
[c] Clear cell (n = 2) Carcinosarcoma (*n* = 6) Neuroendocrine (n = 1).
[d] Only endometroid tumors (n = 77) (missing grade information for n = 1).

.043) and T ($p = .035$). Patients with AR positive tumors also had higher levels of E1 and E2 ($p = .029$ and $p = .020$). In line with these results, mean values of steroid levels were higher in ER-positive tumors than in ER-negative tumors, but not to the level of statistical significance.

3.4. Differences in plasma levels of steroids are reflected in tumor mRNA expression patterns

Gene set expression analysis within the endometrioid subgroup revealed gene sets related to E2F targets, Myc targets and cell cycle/mitotic events to be enriched in patients with low levels of 17-OHP or 11-DOC (Table 4). In patients with high levels of steroids, genes corresponding to gene sets for inflammatory pathways and estrogen signaling were enriched. Overall, differences in gene expression were more pronounced when separating groups by level of 17-OHP, than for any of the other steroids.

For patients with high plasma estrogen levels (top tertile vs bottom tertile) several genes linked to estrogen signaling were differentially expressed (fold change >2), including *PGR* (encoding progesterone receptor) (Supplementary Table S6). GSEA identified two estrogen response gene sets indicating increased ESR1 induced signaling among the top ranked gene sets for high levels of E1 and E2 (Table 4).

4. Discussion

Identification of new cancer biomarkers in blood samples is attractive due to ease of sampling and readily available analysis methods, yet few blood-based biomarkers are validated and in clinical use. We here investigate if a standard method for measuring levels of selected steroids, implemented at our institution, can detect variations in steroid levels in subgroups of endometrial cancer patients, and if these steroids have prognostic value as preoperative biomarkers. This is, to our

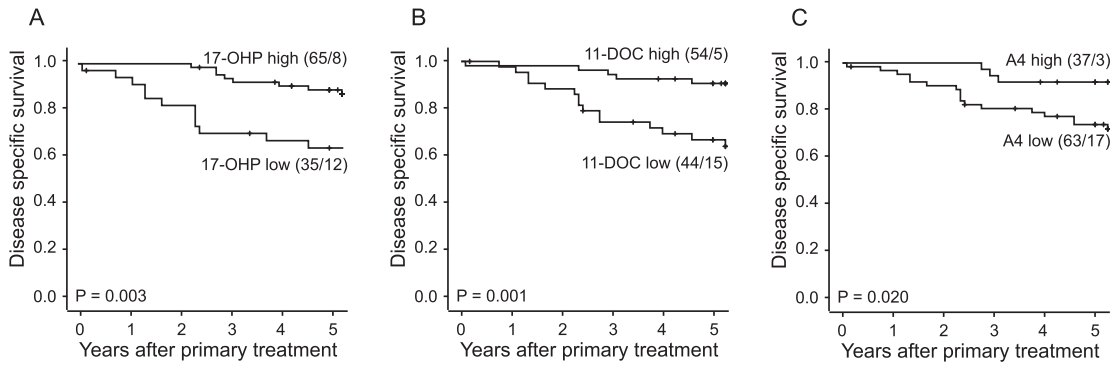


Fig. 2. Univariate survival analysis of endometrial cancer patients grouped by level of A) 17OH-Progesterone (17-OHP) B)11-Deoxycortisol (11-DOC) C) Androstenedione (A4). Cutoffs set by ROC-curve analysis for best prediction of survival at 5 years.

Table 2
Survival analysis by Cox proportional hazards regression model.

Variable	Unadjusted HR	95% CI	p	Adjusted HR[a]	95% CI	p
High preoperative risk[b]	5.12	1.96–13.35	**0.001**			
Low[c] 17OH-Progesterone	3.51	1.43–8.60	**0.006**	2.69	1.09–6.68	**0.033**
Low[c] 11-Deoxycortisol	4.49	1.63–12.38	**0.004**	3.40	1.21–9.51	**0.020**
Low[c] Androstenedione	3.86	1.13–13.16	**0.031**	2.98	0.87–10.26	0.080

HR: hazard ratio, CI: confidence interval.
[a] Adjusted for preoperative risk.
[b] Preoperative risk high: grade 3 endometroid and all non-endometroid histologies.
[c] Cutoff set by ROC-curve analysis for prediction of 5-year survival.

knowledge, the first study indicating prognostic value of circulating levels of 17-OHP, 11-DOC and A4 in endometrial cancer.

Currently, treatment decisions for endometrial cancer are based on a preoperative histopathological evaluation of tumor biopsies in combination with available preoperative imaging. However, risk stratification based on preoperative information is not always accurate, and can result in recurrent disease in putative low-risk patients. A recent meta-analysis found overall pooled agreement in 67%, between preoperative and postoperative histology [32], finding the highest disagreement for endometrioid grade 2 tumors, where omitting lymphadenectomy at surgery is an option. For imaging, size is generally the limiting factor, resulting in low sensitivity when lesions are small. For lymph node metastasis PET/CT is regarded as the optimal radiological modality, but still with limitations in sensitivity (around 70%) [33]. Sentinel node biopsy is emerging as clinical standard in many centres, with sensitivity for metastases at 96% [34], but prolongs surgery in low-risk cases, is not available in all institutions and not applicable in cases where hysterectomy might be avoided, for example in the morbidly obese. In this context, a blood sample providing additional information could be valuable and is easy to add to existing algorithms. It is not unlikely that, as in ongoing initiatives [7,8], a panel combining several markers will provide a useful clinical tool in the future. Our findings point to a role for several steroids as preoperative biomarkers in endometrial cancer, either alone or in combination with other biomarkers.

In our study, low levels of 17-OHP, 11-DOC and A4 predicted poor survival, 17-OHP and 11-DOC independently of the preoperative histological risk assessment, implying that their quantification could provide additional information to clinical algorithms currently in use. We observed an overall association between low levels of these steroids and aggressive characteristics of endometrial cancer. Interestingly, we found that low 17-OHP associates significantly with lymph node metastasis and deep myometrial infiltration in the endometrial subgroup. This might point to a potential role for this steroid in selecting patients for lymphadenectomy or adjuvant treatment also for patients with presumed low-risk disease, which should be addressed in future research.

17-OHP, 11-DOC and A4 are all steroid hormones with sex-steroid related activity. 17-OHP is a weak gestagen, A4 is its derivative with androgenic properties and the precursor of testosterone and estrogen compounds. 11-DOC is another derivative of 17-OHP with glucocorticoid properties. As circulating hormones, they contribute to the environment in which the tumor is evolving and may affect the tumor through activation of the respective hormone receptors [14,26,27,35]. In addition, local intracrinological activity in the tumor or surrounding stroma may contribute to transforming these compounds into more potent signals [23,36].

Gene expression analysis and hormone receptor status in the tumor samples point toward more estrogen associated signaling in patients with higher levels of 17-OHP, 11-DOC and A4. Although unopposed estrogen is an ascertained major risk factor for endometrial cancer, high estrogen signaling in the tumor, reflected in PR expression in endometrial tumors is associated with high differentiation and less aggressiveness [14,37,38]. Our study contributes to this understanding by implying a link between the host endocrine environment and prognostically favorable gene- and protein expression patterns in the tumor.

Although obesity is strongly connected to estrogen levels and consequently to the development of endometrial cancer (recently reviewed in [39]), we found no correlation between the levels of 17-OHP, 11-DOC or A4 and total body fat or fat distribution. However, the observation that BMI and CT-generated fat distribution variables are linked to estrogen levels supports the current understanding of the obesity-estrogen pathogenesis pathway of endometrial cancer [20,40]. A recent study showed that SAV (subcutaneous fat) was the most important contributor to plasma estrogen level [41], whereas visceral fat volume percentage (of total fat volume) has been shown by our group to be associated with poor prognosis in endometrial cancer [42]. In our previous study, visceral fat percentage alone correlated to high estrogen levels in patients with non-endometrial histology [23]. This finding was not confirmed by the present study, which we believe might be due to differences in patient cohorts. Estrogen production is only one of several mechanisms through which fatty tissue affects endometrial cancer pathogenesis [39], and exploring the impact of other factors such as inflammatory activity and insulin metabolism in relation to anatomical fat distribution may provide better insight.

Due to cyclic variations, assessment of hormonal levels in pre-and perimenopausal women are challenging. In the present study, we have focused on postmenopausal women, constituting the majority of endometrial cancer patients. With careful planning and stratification, future trials could explore hormonal variation, fat distribution and endometrial cancer prognosis in younger women to complete the picture.

Table 3
Correlations (Spearmans rank coefficient, ρ) between plasma levels of steroids and measurements of body fat and fat distribution.

	17OH-Progesterone	11-Deoxycortisol	Androstenedione	Testosterone	Estrone	Estradiol
Body Mass Index	0.04	0.03	0.11	0.23*	0.53**	0.63**
Waist circumference	−0.02	−0.04	0.06	0.18	0.50**	0.59**
TAV (CT)	0.12	0.08	0.12	0.19	0.52**	0.61**
SAV (CT)	0.12	0.08	0.16	0.22*	0.51**	0.60**
VAV (CT)	0.03	0.05	0.03	0.10	0.46**	0.54**
VAV%	0.08	−0.01	−0.16	−0.14	−0.01	0.01

TAV: total abdominal fat volume, SAV: subcutaneous abdominal fat volume, VAV: visceral abdominal fat volume, VAV%: VAV/TAV, CT: obtained from CT image.
* Correlation significant at the 0.05 level.
** Correlation significant at the 0.001 level.

Table 4
Gene set expression analysis: Significantly enriched Hallmark genesets (MSigDB) comparing gene expression patterns in endometroid endometrial tumors, grouped by level of steroid hormones. Showing gene sets associated to inflammation, estrogen signaling, mitosis and cell cycle progression. Complete list of significantly enriched gene sets in supplementary table S5.

Enriched gene sets in high plasma levels	17OH-Progesterone[a]		11-Deoxycortisol[a]		Androstenedione[a]		Estrone[b]		Estradiol[b]	
	Rank	FDR	Rank	FDR	Rank	FDR	Rank	FDR	Rank	FDR
TNFA_SIGNALING_VIA_NFKB	1	<0.001	1	<0.001						
IL6_JAK_STAT3_SIGNALING	2	<0.001	3	<0.001	5	0.27				
INFLAMMATORY_RESPONSE	3	<0.001	5	<0.001	6	0.22				
IFN_ALPHA_RESPONSE	4	<0.001			1	<0.001				
IFN_GAMMA_RESPONSE	5	<0.001			2	<0.001				
ALLOGRAFT_REJECTION	6	<0.001			3	<0.001				
ESTROGEN_RESPONSE_EARLY	7	0.03	6	0.05	4	0.33	1	1.33	3	1.03
ESTROGEN_RESPONSE_LATE	8	0.02			8	0.88	2	1.99	6	0.71
Enriched gene sets in low plasma levels	**17OH-Progesterone[a]**		**11-Deoxycortisol[a]**		**Androstenedione[a]**		**Estrone[b]**		**Estradiol[b]**	
	Rank	FDR	Rank	FDR	Rank	FDR	Rank	FDR	Rank	FDR
E2F_TARGETS	1	<0.001	1	<0.001			1	0.83		
MYC_TARGETS_V1	2	<0.001	3	<0.001						
G2M_CHECKPOINT	3	<0.001	2	<0.001			2	0.11		
MYC_TARGETS_V2	5	0.11								
MITOTIC_SPINDLE	6	0.11	4	0.14						
TNFA_SIGNALING_VIA_NFKB							1	0.14	1	1.87

[a] Cutoff determined by ROC analysis for prediction of disease specific survival.
[b] Highest vs. lowest tertile.

In addition, the relevance of endogenous hormone levels for the response to hormonal treatment in endometrial cancer should be explored. For young obese women with endometrial cancer, often presenting with low grade endometrioid endometrial cancers, results from such research could lead to important improvements in current treatment strategies.

We have employed LC-MS/MS protocols in clinical use to evaluate steroid hormones in endometrial cancer patients, providing reliable and accurate estimates of plasma levels. The samples have been stored in our biobank in −80 degrees for 4 to 9 years prior to analysis, making possible degradation an issue in the interpretation of the results. Definitive data supporting reliability of steroid levels after long-time storage is lacking. Studies attempting to address this question have generally found small changes in levels over time, with close to unchanged ranking of samples [43,44]. Analysis of our data does not demonstrate any obvious changes due to storage time, but ultimately the retrospective nature of the study makes complete exclusion of this bias difficult. To validate our results a prospective inclusion of patients with standardized sampling and immediate analysis is needed.

In conclusion, our study shows that preoperative endogenous steroid levels are associated with outcome in endometrial cancer. Our findings imply that endogenous steroids are not merely mirroring circulating estrogen levels or obesity, and provide additional prognostic information to preoperative tumor histologic assessment. Measuring circulating steroid hormones is easy and low-cost and can potentially be included in treatment algorithms, however the proper cutoffs and applications need to be further elucidated and validated in future trials.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2019.11.123.

Acknowledgement

We thank Kadri Madisoo and Olivera Bozickovic for technical assistance.

Funding

This study was supported by Helse Vest (HV 440088), the University of Bergen, the Norwegian Cancer Society (190202), the Research Council of Norway (273280) and Bergen Research Foundation.

Declaration of competing interest

There are no conflicts of interest to disclose.

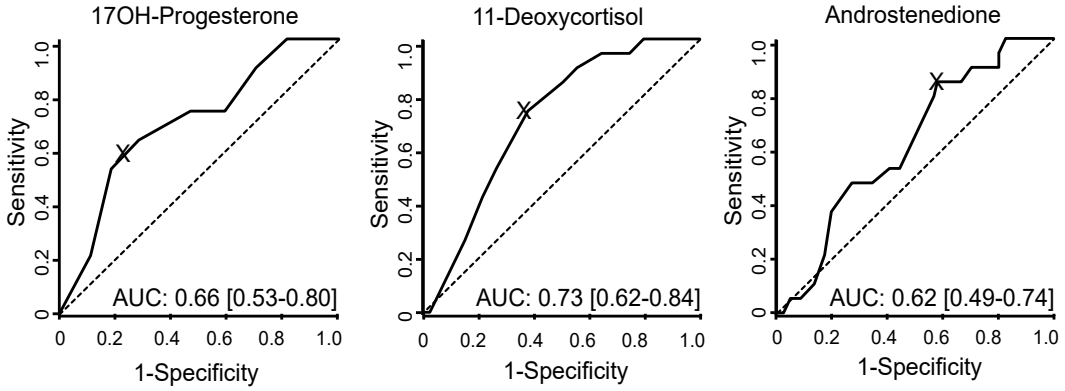
Author contributions

I.L.T, D.F and C·K conceived of and planned the project. I.L.T, M.K·H, K.E.F, K·V, B.A, B-E.B, I.S·H, J.T and C·K contributed to collection of samples and clinical data. D.F, I.L.T and C·K analyzed and interpreted the results. D.F, I.L.T and C·K took the lead in writing the manuscript. C.K. supervised the project. All authors helped shape the analysis and manuscript through critical feedback.

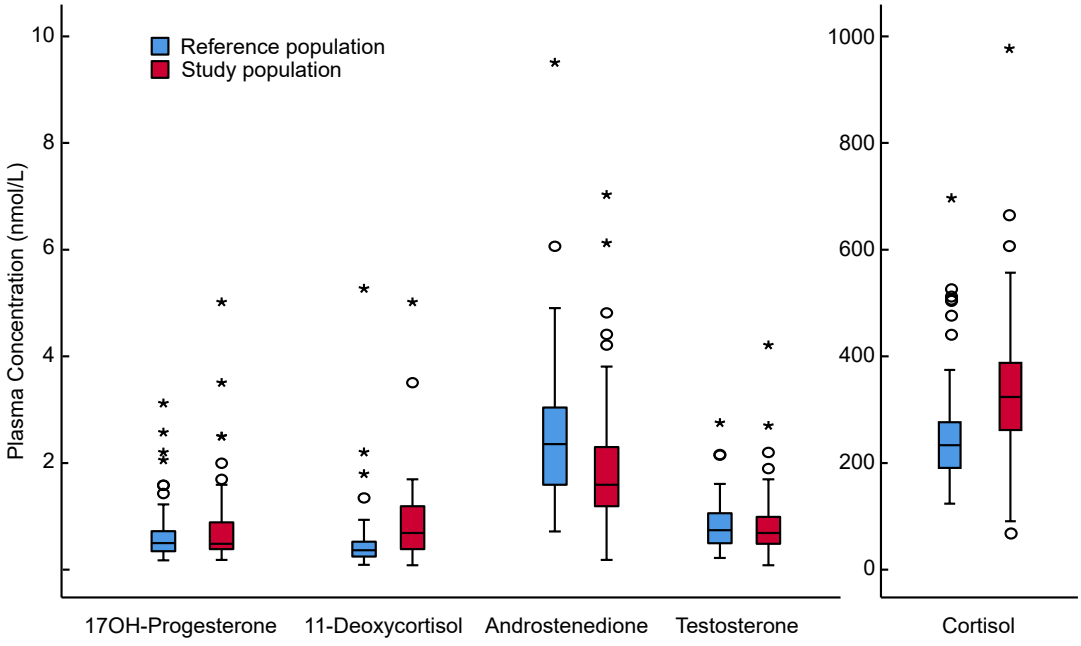
References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 68 (2018) 394–424.
[2] P. Morice, A. Leary, C. Creutzberg, N. Abu-Rustum, E. Darai, Endometrial cancer, Lancet (London, England) 387 (2016) 1094–1108.
[3] F. Amant, P. Moerman, P. Neven, D. Timmerman, E. Van Limbergen, I. Vergote, Endometrial cancer, Lancet (London, England) 366 (2005) 491–505.
[4] T. Odagiri, H. Watari, M. Hosaka, T. Mitamura, Y. Konno, T. Kato, et al., Multivariate survival analysis of the patients with recurrent endometrial cancer, J. Gynecol. Oncol. 22 (2011) 3–8.
[5] J. Trovik, K.K. Mauland, H.M. Werner, E. Wik, H. Helland, H.B. Salvesen, Improved survival related to changes in endometrial cancer treatment, a 30-year population based perspective, Gynecol. Oncol. 125 (2012) 381–387.
[6] H.M. Werner, H.B. Salvesen, Current status of molecular biomarkers in endometrial cancer, Curr. Oncol. Rep. 16 (2014) 403.
[7] A. Auguste, C. Genestie, M. De Bruyn, J. Adam, A. Le Formal, F. Drusch, et al., Refinement of high-risk endometrial cancer classification using DNA damage response biomarkers: a TransPORTEC initiative, Mod. Pathol. 31 (2018) 1851–1861.
[8] S. Kommoss, M.K. McConechy, F. Kommoss, S. Leung, A. Bunz, J. Magrill, et al., Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series, Annals of oncology: official journal of the European Society for Medical Oncology 29 (2018) 1180–1188.
[9] C. Reijnen, N.C. Visser, J.C. Kasius, D. Boll, P.M. Geomini, H. Ngo, et al., Improved preoperative risk stratification with CA-125 in low-grade endometrial cancer: a multicenter prospective cohort study, J. Gynecol. Oncol. 30 (2019) e70.
[10] D.J. Brennan, A. Hackethal, A.M. Metcalf, J. Coward, K. Ferguson, M.K. Oehler, et al., Serum HE4 as a prognostic marker in endometrial cancer–a population based study, Gynecol. Oncol. 132 (2014) 159–165.
[11] A. Di Cello, M. Di Sanzo, F.M. Perrone, G. Santamaria, E. Rania, E. Angotti, et al., DJ-1 is a reliable serum biomarker for discriminating high-risk endometrial cancer, Tumour Biol. 39 (2017), 1010428317705746.
[12] H. Engerud, K. Hope, H.F. Berg, K.E. Fasmer, I.L. Tangen, I.S. Haldorsen, et al., Plasma growth differentiation factor-15 is an independent marker for aggressive disease in endometrial cancer, PLoS One 14 (2019), e0210585.

[13] E. Wik, M.B. Raeder, C. Krakstad, J. Trovik, E. Birkeland, E.A. Hoivik, et al., Lack of estrogen receptor-alpha is associated with epithelial-mesenchymal transition and PI3K alterations in endometrial carcinoma, Clinical cancer research: an official journal of the American Association for Cancer Research 19 (2013) 1094–1105.
[14] I.L. Tangen, H.M. Werner, A. Berg, M.K. Halle, K. Kusonmano, J. Trovik, et al., Loss of progesterone receptor links to high proliferation and increases from primary to metastatic endometrial cancer lesions, European journal of cancer (Oxford, England: 1990) 50 (2014) 3003–3010.
[15] Y. Zhang, D. Zhao, C. Gong, F. Zhang, J. He, W. Zhang, et al., Prognostic role of hormone receptors in endometrial cancer: a systematic review and meta-analysis, World Journal of Surgical Oncology 13 (2015) 208.
[16] N.E. Allen, T.J. Key, L. Dossus, S. Rinaldi, A. Cust, A. Lukanova, et al., Endogenous sex hormones and endometrial cancer risk in women in the European prospective investigation into Cancer and nutrition (EPIC), Endocr. Relat. Cancer 15 (2008) 485–497.
[17] E. Audet-Walsh, J. Lepine, J. Gregoire, M. Plante, P. Caron, B. Tetu, et al., Profiling of endogenous estrogens, their precursors, and metabolites in endometrial cancer patients: association with risk and relationship to clinical characteristics, J. Clin. Endocrinol. Metab. 96 (2011) E330–E339.
[18] L.A. Brinton, B. Trabert, G.L. Anderson, R.T. Falk, A.S. Felix, B.J. Fuhrman, et al., Serum estrogens and estrogen metabolites and endometrial Cancer risk among postmenopausal women, Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 25 (2016) 1081–1089.
[19] A. Lukanova, E. Lundin, A. Micheli, A. Arslan, P. Ferrari, S. Rinaldi, et al., Circulating levels of sex steroid hormones and risk of endometrial cancer in postmenopausal women, Int. J. Cancer 108 (2004) 425–432.
[20] H. Austin, J.M. Austin, E.E. Partridge, K.D. Hatch, H.M. Shingleton, Endometrial Cancer, obesity, and body fat distribution, Cancer Res. 51 (1991) 568.
[21] N. Potischman, R.N. Hoover, L.A. Brinton, P. Siiteri, J.F. Dorgan, C.A. Swanson, et al., Case-control study of endogenous steroid hormones and endometrial cancer, J. Natl. Cancer Inst. 88 (1996) 1127–1135.
[22] K.A. Michels, L.A. Brinton, N. Wentzensen, K. Pan, C. Chen, G.L. Anderson, et al., Postmenopausal androgen metabolism and endometrial Cancer risk in the Women's Health Initiative observational study, JNCI Cancer Spectrum 3 (2019).
[23] I.L. Tangen, K.E. Fasmer, G.F. Konings, A. Jochems, B. Delvoux, S. Xanthoulea, et al., Blood steroids are associated with prognosis and fat distribution in endometrial cancer, Gynecol. Oncol. 152 (2019) 46–52.
[24] J. Trovik, E. Wik, I.M. Stefansson, J. Marcickiewicz, S. Tingulstad, A.C. Staff, et al., Stathmin overexpression identifies high-risk patients and lymph node metastasis in endometrial cancer, Clinical cancer research: an official journal of the American Association for Cancer Research 17 (2011) 3368–3377.
[25] C. Krakstad, J. Trovik, E. Wik, I.B. Engelsen, H.M.J. Werner, E. Birkeland, et al., Loss of GPER identifies new targets for therapy among a subgroup of ERα-positive endometrial cancer patients with poor outcome, Br. J. Cancer 106 (2012) 1682–1688.
[26] I.L. Tangen, J.T. Veneris, M.K. Halle, H.M. Werner, J. Trovik, L.A. Akslen, et al., Expression of glucocorticoid receptor is associated with aggressive primary endometrial cancer and increases from primary to metastatic lesions, Gynecol. Oncol. 147 (2017) 672–677.
[27] I.L. Tangen, T.B. Onyango, R. Kopperud, A. Berg, M.K. Halle, A.M. Øyan, et al., Androgen receptor as potential therapeutic target in metastatic endometrial cancer, Oncotarget 7 (2016) 49289–49298.
[28] P. Methlie, S.S. Hustad, R. Kellmann, B. Almås, M.M. Erichsen, E. Husebye, et al., Multisteroid LC-MS/MS assay for glucocorticoids and androgens, and its application in Addison's disease, Endocrine connections 2 (2013) 125–136.
[29] Bertelsen BE, Kellmann R, Viste K, Bjørnevik A, Eikesdal H, Lønning P, et al. An Ultrasensitive Routine LC-MS/MS-Method for Estradiol and Estrone in the Clinically Relevant Sub-Picomolar Range. (Manuscript submitted).
[30] A. Liberzon, C. Birger, H. Thorvaldsdóttir, M. Ghandi, P. Mesirov Jill, P. Tamayo, The molecular signatures database Hallmark gene set collection, Cell Systems 1 (2015) 417–425.
[31] G. Eisenhofer, M. Peitzsch, D. Kaden, K. Langton, C. Pamporaki, J. Masjkur, et al., Reference intervals for plasma concentrations of adrenal steroids measured by LC-MS/MS: impact of gender, age, oral contraceptives, body mass index and blood pressure status, Clinica chimica acta; international journal of clinical chemistry 470 (2017) 115–124.
[32] N.C.M. Visser, C. Reijnen, L. Massuger, I.D. Nagtegaal, J. Bulten, J.M.A. Pijnenborg, Accuracy of endometrial sampling in endometrial carcinoma: a systematic review and meta-analysis, Obstet. Gynecol. 130 (2017) 803–813.
[33] V.R. Bollineni, S. Ytre-Hauge, O. Bollineni-Balabay, H.B. Salvesen, I.S. Haldorsen, High diagnostic value of 18F-FDG PET/CT in endometrial Cancer: systematic review and meta-analysis of the literature, J. Nucl. Med. 57 (2016) 879–885.
[34] A.J. Bodurtha Smith, A.N. Fader, E.J. Tanner, Sentinel lymph node assessment in endometrial cancer: a systematic review and meta-analysis, Am. J. Obstet. Gynecol. 216 (2017) 459–476(e10).
[35] J.M. Vahrenkamp, C.-H. Yang, A.C. Rodriguez, A. Almomen, K.C. Berrett, A.N. Trujillo, et al., Clinical and genomic crosstalk between glucocorticoid receptor and estrogen receptor α in endometrial Cancer, Cell Rep. 22 (2018) 2995–3005.
[36] G. Konings, L. Brentjens, B. Delvoux, T. Linnanen, K. Cornel, P. Koskimies, et al., Intracrine regulation of estrogen and other sex steroid levels in endometrium and non-gynecological tissues; pathology, physiology, and drug discovery, Front. Pharmacol. 9 (2018) 940.
[37] H.B. Salvesen, I.S. Haldorsen, J. Trovik, Markers for individualised therapy in endometrial carcinoma, The Lancet Oncology 13 (2012) e353–e361.
[38] A.C. Rodriguez, Z. Blanchard, K.A. Maurer, J. Gertz, Estrogen signaling in endometrial Cancer: a Key oncogenic pathway with several open questions, Hormones & cancer 10 (2019) 51–63.
[39] E. Shaw, M. Farris, J. McNeil, C. Friedenreich, Obesity and endometrial Cancer, Recent results in cancer research Fortschritte der Krebsforschung Progres dans les recherches sur le cancer 208 (2016) 107–136.
[40] H. Oh, S.B. Coburn, C.E. Matthews, R.T. Falk, E.S. LeBlanc, J. Wactawski-Wende, et al., Anthropometric measures and serum estrogen metabolism in postmenopausal women: the Women's Health Initiative observational study, Breast Cancer Res. 19 (2017) 28.
[41] W.J. van Weelden, K.E. Fasmer, I.L. Tangen, J. IntHout, K. Abbink, A.E. van Herwaarden, et al., Impact of body mass index and fat distribution on sex steroid levels in endometrial carcinoma: a retrospective study, BMC Cancer 19 (2019) 547.
[42] K.K. Mauland, O. Eng, S. Ytre-Hauge, I.L. Tangen, A. Berg, H.B. Salvesen, et al., High visceral fat percentage is associated with poor outcome in endometrial cancer, Oncotarget 8 (2017) 105184–105195.
[43] G. Bolelli, P. Muti, A. Micheli, R. Sciajno, F. Franceschetti, V. Krogh, et al., Validity for epidemiological studies of long-term cryoconservation of steroid and protein hormones in serum and plasma, Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 4 (1995) 509–513.
[44] K. Holl, E. Lundin, M. Kaasila, K. Grankvist, Y. Afanasyeva, G. Hallmans, et al., Effect of long-term storage on hormone measurements in samples from pregnant women: the experience of the Finnish maternity cohort, Acta oncologica (Stockholm, Sweden) 47 (2008) 406–412.



Supplementary Fig. S1. Receiver operating characteristic curve of steroid hormone for prediction of disease-specific death within 5 years of follow up. Optimal cutoffs (X) are calculated by the Youden Index



Supplementary Fig. S2. Comparison of plasma steroid levels in study cohort with a LC-MS/MS reference cohort [31]. Boxes represent median values with interquartile ranges, whiskers represent non-outlying max and min values (<1.5 times the interquartile range).

Supplementary Table S1. Clinical-pathological characteristics for included patients compared to the prospective cohort.

Variable	Study cohort (n=100)	Prospective cohort (n=1038)	p-value
Age			0.19 (Mann-Whitney U)
Median [range]	69[44]	66[69]	
Parity			0.98 (Chi-square)
0	16 %	16 %	
≥1	84 %	83 %	
BMI			0.54 (t-test)
Mean (SD)	28 (5.5)	28(6.8)	
Primary treatment			0.04 (Chi-square)
Hysterectomy	100 %	94 %	
Curettage only	0 %	5 %	
Adjuvant treatment			0.26 (Chi-square)
None	70 %	68 %	
Chemotherapy	26 %	20 %	
Radiotherapy	3 %	8 %	
Chemoradiotherapy	0 %	1 %	
Hormonal therapy	1 %	2 %	
5-year survival	80 %	81 %	0.87 (Mantel-Cox log-rank)
FIGO-09 stage			0.44 (Chi-square)
I	77 %	74 %	
II	10 %	8 %	
III	10 %	12 %	
IV	3 %	7 %	
Histologic type			0.89 (Chi-square)
Endometrioid	77 %	78 %	
Non-endometrioid	23 %	22 %	
Histologic grade			0.11 (Chi-square)
Grade 1/2	56 %	63 %	
Grade 3	43 %	34 %	
Lymph node status			0.43 (Chi-square)
Negative	79 %	63 %	
Positive	8 %	9 %	
No LA	13 %	28 %	
Myometrial infiltration			0.06 (Chi-square)
<50%	53 %	59 %	
≥50%	47 %	35 %	

Percentages not accounted for represent missing values

LA: Lymphadenectomy, EEC: Endometrioid endometrial cancer

Supplementary Table S2. Correlations (spearmans rank coefficient, ρ) between levels of steroid hormones within patients, and storage time (−80 degrees C)

	17OH-Progesterone	11-Deoxycortisol	Andro-stenedione	Testosterone	Estrone	Estradiol
17OH-Progesterone		0.72**	0.60**	0.54**	0.40**	0.43**
11-Deoxycortisol			0.59**	0.34**	0.16	0.15
Androstenedione				0.60**	0.53**	0.46**
Testosterone					0.47**	0.51**
Estrone						0.92**
Storage time	-0.02	-0.01	-0.05	0.08	0.03	0.06

* Correlation significant at the 0.05 level ** Correlation significant at the 0.001 level

Supplementary Table S3. Clinical-pathological characteristics related to median steroid hormone levels (10–90 percentile) in endometrioid endometrial cancer patients.

	17OH-Progesterone n=77		11-Deoxycortisol n=75		Androstenedione n=77		Testosterone n=77		Estrone n=73		Estradiol n=73	
	nmol/L	p	nmol/L	p	nmol/L	p	nmol/L	p	pmol/L	p	pmol/L	p
FIGO-09 stage		**0.014**		0.086		**0.028**		0.078		0.379		0.41
I-II (n=70)	0.6 (0.3-1.4)		0.8 (0.3-1.6)		1.7 (0.9-3.6)		0.8 (0.4-1.6)		107 (46-218)		22 (8-66)	
III-IV (n=7)	0.3 (0.2-0.5)		0.5 (0.2-1.0)		1.1 (0.8-1.6)		0.5 (0.2-0.9)		75 (41-117)		22 (6-32)	
Histologic grade		0.345		0.775		0.194		0.079		**0.012**		**0.015**
Grade 1/2 (n=56)	0.6 (0.2-1.8)		0.8 (0.3-1.6)		1.7 (0.9-3.9)		0.8 (0.4-1.6)		108 (50-243		24 (10-104)	
Grade 3 (n=20)	0.6 (0.2-1.1)		0.9 (0.3-1.7)		1.6 (0.8-2.8)		0.7 (0.2-1.5)		72 (41-134)		15 (5-40)	
Lymph node status		**0.029**		0.34		0.163		**0.025**		0.249		0.176
Negative (n=63)	0.6 (0.2-1.6)		0.8 (0.3-1.6)		1.7 (0.9-3.7)		0.8 (0.4-1.6)		107 (47-216)		23 (9-68)	
Positive (n=4)	0.4 (0.2-0.5)		0.6 (0.2-1.1)		1.4 (1.0-1.6)		0.5 (0.3-0.6)		75 (50-100)		16 (6-22)	
Myometrial infiltr.		**0.028**		0.087		0.175		0.413		0.092		0.067
<50% (n=43)	0.7 (0.2-1.5)		0.9 (0.3-1.7)		1.9 (1.0-4.0)		0.8 (0.4-1.6)		110 (54-276)		23 (11-141)	
≥50% (n=34)	0.5 (0.2-1.3)		0.7 (0.3-1.4)		1.6 (0.9-3.6)		0.7 (0.3-1.5)		82 (39-193)		17 (6-53)	

Statistical comparisons are done with Mann-Whitney U test for independent samples.

Supplementary Table S4. Comparison of median steroid hormone levels (10–90 percentile) between patients grouped by hormone receptor expression pattern of the tumor.

	17OH-Progesterone n=100		11-Deoxycortisol n=98		Androstenedione n=100		Testosterone n=100		Estrone n=96		Estradiol n=96	
	nmol/L	p	nmol/L	p	nmol/L	p	nmol/L	p	pmol/L	p	pmol/L	p
PR		**0.043**		0.20		0.21		**0.035**		**0.002**		**<0.001**
Positive (n=67)	0.6 (0.3-1.2)		0.8 (0.3-1.5)		1.6 (0.9-3.6)		0.8 (0.4-1.5)		107 (50-218)		24 (10-67)	
Negative (n=27)	0.5 (0.2-1.2)		0.6 (0.3-1.6)		1.6 (0.7-3.6)		0.6 (0.3-1.3)		64 (28-177)		11 (5-46)	
ER		0.11		0.30		0.20		0.23		0.16		0.08
Positive (n=64)	0.6 (0.2-1.5)		0.7 (0.3-1.6)		1.6 (0.9-3.6)		0.8 (0.4-1.5)		104 (47-217)		23 (9-65)	
Negative (n=29)	0.5 (0.2-1.0)		0.6 (0.3-1.6)		1.3 (0.8-3.6)		0.7 (0.3-1.2)		72 (31-237)		16 (6-52)	
AR		0.07		0.58		0.26		0.06		**0.029**		**0.02**
Positive (n=51)	0.7 (0.2-1.5)		0.8 (0.3-1.56)		1.6 (0.9-3.7)		0.8 (0.4-1.5)		114 (48-217)		24 (10-69)	
Negative (n=36)	0.5 (0.2-1.0)		0.7 (0.3-1.6)		1.6 (0.8-3.6)		0.7 (0.3-1.3)		71 (36-238)		17 (5-50)	
GR		0.30		0.60		0.50		0.45		0.24		0.22
Positive (n=59)	0.6 (0.2-1.0)		0.7 (0.3-1.5)		1.6 (0.9-3.7)		0.8 (0.4-1.4)		100 (45-237)		23 (9-59)	
Negative (n=17)	0.5 (0.2-1.9)		0.6 (0.3-2.4)		1.6 (0.8-2.8)		0.7 (0.3-1.3)		85 (49-140)		16 (9-41)	

Statistical comparisons are done with Mann-Whitney U test for independent samples.

Supplementary Table S5. Gene set expression analysis: Top ranked Hallmark genesets (MSigDB) comparing gene expression patterns in endometroid endometrial cancer tumors, grouped by level of steroid hormones. Results with false detection rate (FDR) > 2% not shown.

Rank	17OH-Progesterone high[1]	FDR	17OH-Progesterone low[1]	FDR
1	TNFA_SIGNALING_VIA_NFKB	<0.001	E2F_TARGETS	<0.001
2	IL6_JAK_STAT3_SIGNALING	<0.001	MYC_TARGETS_V1	<0.001
3	INFLAMMATORY_RESPONSE	<0.001	G2M_CHECKPOINT	<0.001
4	IFN_ALPHA_RESPONSE	<0.001	OXIDATIVE_PHOSPHORYLATION	0.01
5	IFN_GAMMA_RESPONSE	<0.001	MYC_TARGETS_V2	0.11
6	ALLOGRAFT_REJECTION	<0.001	MITOTIC_SPINDLE	0.11
7	ESTROGEN_RESPONSE_EARLY	0.03	DNA_REPAIR	0.67
8	ESTROGEN_RESPONSE_LATE	0.02		
9	COAGULATION	0.02		
10	APOPTOSIS	0.48		
11	COMPLEMENT	0.61		
12	IL2_STAT5_SIGNALING	0.64		
13	KRAS_SIGNALING_UP	0.62		
14	ANGIOGENESIS	0.78		
15	ANDROGEN_RESPONSE	1.25		
16	HYPOXIA	1.6		

Rank	11-Deoxycortisol high[1]	FDR	11-Deoxycortisol low[1]	FDR
1	TNFA_SIGNALING_VIA_NFKB	<0.001	E2F_TARGETS	<0.001
2	HYPOXIA	<0.001	G2M_CHECKPOINT	<0.001
3	IL6_JAK_STAT3_SIGNALING	<0.001	MYC_TARGETS_V1	<0.001
4	EMT	<0.001	MITOTIC_SPINDLE	0.14
5	INFLAMMATORY_RESPONSE	<0.001	OXIDATIVE_PHOSPHORYLATION	0.19
6	ESTROGEN_RESPONSE_EARLY	0.05	FATTY_ACID_METABOLISM	0.82
7	ROS_PATHWAY	0.05	PROTEIN_SECRETION	1.45
8	ANGIOGENESIS	0.1		
9	APOPTOSIS	0.09		
10	COAGULATION	0.19		
11	INTERFERON_GAMMA_RESPONSE	0.31		
12	INTERFERON_ALPHA_RESPONSE	0.48		
13	P53_PATHWAY	0.58		
14	GLYCOLYSIS	0.88		
15	ALLOGRAFT_REJECTION	1.24		

Rank	Androstenedione high[1]	FDR	Androstenedione low[1]	FDR
1	IFN_ALPHA_RESPONSE	<0.001	MYOGENESIS	1.91
2	IFN_GAMMA_RESPONSE	<0.001		
3	ALLOGRAFT_REJECTION	<0.001		
4	ESTROGEN_RESPONSE_EARLY	0.33		
5	IL6_JAK_STAT3_SIGNALING	0.27		
6	INFLAMMATORY_RESPONSE	0.22		
7	E2F_TARGETS	0.7		
8	ESTROGEN_RESPONSE_LATE	0.88		
9	ROS_PATHWAY	1.91		

Rank	Estrone High[2]	FDR	Estrone low[2]	FDR
1	ESTROGEN_RESPONSE_EARLY	1.33	TNFA_SIGNALING_VIA_NFKB	0.14
2	ESTROGEN_RESPONSE_LATE	1.99	E2F_TARGETS	0.83
3			MYOGENESIS	1.9
5			P53_PATHWAY	1.7
Rank	**Estradiol high[2]**	**FDR**	**Estradiol low[2]**	**FDR**
1	PROTEIN_SECRETION	0.48	TNFA_SIGNALING_VIA_NFKB	1.87
2	UNFOLDED_PROTEIN_RESPONSE	1.55		
3	ESTROGEN_RESPONSE_LATE	1.03		
4	FATTY_ACID_METABOLISM	0.82		
5	GLYCOLYSIS	0.66		
5	PEROXISOME	0.81		
6	ESTROGEN_RESPONSE_EARLY	0.71		

Gray shading marks inflammation associated gene sets, encased gene sets are related to estrogen signalling.

[1] Cutoff determined by ROC analysis for prediction of disease specific survival

[2] Higest vs. lowest tertile

Supplementary Table S6. Significance Analysis of Micrarray (SAM): Differentially expressed genes in endometrioid endometrial cancer tumors in patients with high plasma estrogen level compared to those with low (top vs bottom tertile). All genes with positive fold change (FC) > 2 and false discovery rate (FDR) < 1% for either E1 or E2 are displayed.

Gene Name	Rank E2	FC E2	FDR E2	Rank E1	FC E1	FDR E1	Relation to estrogen signaling*	Ref.
PGR	1	-5.4	<0.001	6	-4.0	<0.001	Progesterone receptor	Mohammed, H et al. 2015
GALNT4	2	-3.2	<0.001	22	-2.7	<0.001	Estrogen regulated	Wright, P. K. et al. 2009
ANO1	3	-3.4	<0.001	13	-3.0	<0.001	Possible	Yamagata et al. 2016
SLC47A1	4	-7.6	<0.001	3	-7.4	<0.001	Possible	Droog et al. 2017
AL137566/PGR	7	-7.4	<0.001	115	4.6	3.0	Progesterone receptor	Mohammed, H et al. 2015
NUCB2	8	-2.1	<0.001	49	1.9	1.8	Possible	Chung et al. 2015
MMP26	10	-7.5	<0.001	10	-7.0	<0.001	Estrogen regulated	Li et al. 2004
FAM189A2	11	-4.8	<0.001	15	-4.1	<0.001	No apparent relation	
SPINK4	15	-2.2	<0.001	9	2.0	<0.001	Estrogen regulated	Williams et al 2008
SPDEF	19	-3.8	<0.001	135	2.7	3.9	Possible	Fletcher, M. et al. 2013
ELP3	21	-2.4	<0.001	27	-2.0	<0.001	Estrogen regulated	Lorent et al. 2019
CPM	22	-4.6	<0.001	2	-5.6	<0.001	No apparent relation	
GREB1	23	-2.6	<0.001	36	-2.3	<0.001	Estrogen regulated	Mohammed, H et al. 2013
MFAP3L	24	-2.3	<0.001	235	1.7	5.5	No apparent relation	
CN479762 (DIO2)	27	-2.2	<0.001	18	-2.2	<0.001	No apparent relation	
KCNK6	34	-2.3	<0.001	4	-2.4	<0.001	Possible	Patani, N., et al. 2014
DIO2	40	-3.2	<0.001	23	-3.1	<0.001	No apparent relation	
ZNF516	46	-2.2	<0.001	133	1.9	3.9	No apparent relation	
ALKAL2	170	3.6	5.5	21	-4.1	<0.001	No apparent relation	
SMAD9	57	2.1	1.5	29	-2.1	<0.001	No apparent relation	
ANAPC4	51	2.4	1.7	38	-2.3	<0.001	No apparent relation	
ESR1	103	3	4.1	2734	2.0	28.4	Estrogen receptor	

* Relation to estrogen signalling explored through searching Pubmed for the gene name + estrogen and evaluating references.
E2: Estradiol, E1:Estrone

Gynecologic Oncology 160 (2021) 396–404

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Maintained survival outcome after reducing lymphadenectomy rates and optimizing adjuvant treatment in endometrial cancer

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HIGHLIGHTS

- A cohort of 1308 endometrial cancer patients was assessed for outcome related to treatment changes over the last two decades.
- The rate of lymphadenectomy was reduced from approximately 80% to 50% without affecting survival or recurrence rates.
- Omitting adjuvant radiotherapy for a chemotherapy alone policy in high risk patients did not worsen survival or recurrence.

ARTICLE INFO

Article history:
Received 17 October 2020
Accepted 3 December 2020
Available online 13 December 2020

Keywords:
Endometrial cancer
Lymphadenectomy
Chemotherapy
Radiotherapy
Survival

ABSTRACT

Objective. Main controversies in endometrial cancer treatment include the role of lymphadenectomy and optimal adjuvant treatment. We assessed clinical outcome in a population-based endometrial cancer cohort in relation to changes in treatment management over two decades.

Methods. All consenting endometrial cancer patients receiving primary treatment at Haukeland University Hospital from 2001 to 2019 were included (n = 1308). Clinicopathological variables were evaluated for year-to-year changes. Clinical outcome before and after discontinuing adjuvant radiotherapy and individualizing extent of lymphadenectomy was analyzed.

Results. The rate of lymphadenectomy was reduced from 78% in 2001–2012 to 53% in 2013–2019. The rate of patients with verified lymph node metastases was maintained (9% vs 8%, $p = 0.58$) and FIGO stage I patients who did not undergo lymphadenectomy had stable 3-year recurrence-free survival (88% vs 90%, $p = 0.67$). Adjuvant chemotherapy for completely resected FIGO stage III patients increased from 27% to 97% from 2001 to 2009 to 2010–2019, while adjuvant radiotherapy declined from 57% to 0% ($p < 0.001$). These patients had improved 5-year overall- and recurrence-free survival; 0.49 [95% CI: 0.37–0.65] in 2001–2009 compared to 0.61 [0.45–0.83] in 2010–2019, $p = 0.04$ and 0.51 [0.39–0.68] to 0.71 [0.60–0.85], $p = 0.03$, respectively. For stage I, II and IV, survival rates were unchanged.

Conclusions. Our study demonstrates that preoperative stratification by imaging and histological assessments permits a reduction in lymphadenectomy to around 50%, and is achievable without an increase in recurrences at 3 years. In addition, our findings support that adjuvant chemotherapy alone performs equally to adjuvant radiotherapy with regard to survival, and is likely superior in advanced stage patients.

1. Introduction

Endometrial cancer is the most common gynecological cancer in industrialized countries, with a cumulative lifetime risk of 2–3% in women [1,2]. The prognosis in endometrial cancer is generally good due to detection at an early stage where surgery is likely curative [3]. Thus, selecting an appropriate level of treatment that balances the risk of recurrence with the risk of iatrogenic morbidity is a major challenge.

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https://doi.org/10.1016/j.ygyno.2020.12.002

Currently, main controversies include the mode and extent of lymph tissue dissection for staging and selecting optimal adjuvant treatment regimens [4,5]. As an extensive research effort is ongoing to address these topics, oncological centers develop local, national or international guidelines, based on their respective evaluation of scientific evidence, available resources and clinical tradition.

During the last decade, several changes in patient treatment have been implemented for endometrial cancer patients in our region. In 2009 national guidelines were changed; adjuvant radiotherapy (external beam +/− brachytherapy) was no longer recommended for patients with high-risk tumors, defined as FIGO (International Federation of Gynecology and Obstetrics) stage IB grade 3 endometrioid, all stage I non-endometrioid, and completely resected stage II-III [6]. Instead adjuvant platinum based chemotherapy was advocated for all high-risk tumors, motivated by emerging data suggesting better survival outcome when opting for chemotherapy in the adjuvant setting [7]. In 2009 and 2011 respectively, pelvic magnetic resonance imaging (MRI) and fluorodeoxyglucose-positron emission tomography/computed tomography (PET/CT) were gradually integrated in preoperative diagnostics at Haukeland University Hospital. Finally, in October 2015, the MoMaTEC2 study (Molecular Markers in the Treatment of Endometrial Cancer, ClinicalTrials.gov Identifier: NCT02543710) was launched, evaluating the implementation of estrogen and progesterone receptor (ER/PR) expression in preoperative biopsies in combination with histological subtyping and imaging, with the intent to reduce the rate of patients undergoing lymphadenectomy.

The aim of this study was to assess effects and outcome when discontinuing adjuvant radiotherapy and reducing the rate of patients undergoing lymphadenectomy through more extensive preoperative patient stratification. Additionally, we explored trends in clinical and pathological variables that could affect patient outcome during the observed period.

2. Methods

2.1. Ethical considerations

The study was approved by the Western Regional Committee for Medical and Health Research Ethics (REK 2009/2315, 2018/594, and 2019/1020). All included patients signed an informed consent.

2.2. Patient series

Haukeland University Hospital serves approximately 10% of the Norwegian gynecological population as a full-scale gynecological oncology center, providing treatment for all endometrial cancer for local patients. Additionally, the center receives high-risk/advanced stage patients from neighboring counties, comprising approximately 15% of the cohort. The population of Hordaland is demographically representative of the Norwegian population, with similar distributions of age, gender, and body mass index (BMI) [8].

All consenting patients referred to Haukeland University Hospital for primary treatment of endometrial cancer from 2001 to 2019 were included. Patients were surgically staged according to FIGO 2009 criteria; patients treated prior to 2009 were reclassified according to the 2009 criteria as previously described [9]. Clinical and pathological variables were collected from the medical records. Radiological findings were recorded based on the radiology report. The surgery- and multidisciplinary tumor board reports were also reviewed to record how radiological findings had been perceived prior to surgery and to identify reasons for performing or not performing lymphadenectomy. The imaging protocols employed at our institution are largely in line with recommended European guidelines for preoperative imaging in endometrial cancer [10].

2.3. Treatment

Standard treatment was hysterectomy with bilateral salpingo-oophorectomy. Indications for lymphadenectomy changed over the study period (see below). Omentectomy was performed in patients with serous and clear cell tumors. All hysterectomies were performed by laparotomy until the introduction of robotic-assisted laparoscopic hysterectomy in 2010 and conventional laparoscopy in 2013 for selected patients (manageable comorbidity, no presumed extrauterine disease, and para-aortic lymphadenectomy not planned for). In the palliative setting, treatment options included debulking, hysterectomy for symptom control, or primary non-surgical therapy (chemotherapy, radiotherapy, or hormonal therapy). Treatment decisions were made at tumor board meetings including specialists in gynecological oncology, oncology, radiology, and pathology.

2.4. Indications for lymphadenectomy

Indications for lymphadenectomy changed during the observation period from a general pelvic sampling policy (sampling pelvic nodes at the surgeon's discretion unless deemed not tolerable or restricted access perioperatively) to a selective policy based on preoperative risk assessment. Preoperative low-, intermediate-, and high-risk groups were defined by histological assessment of curettage/endometrial biopsy and radiological findings according to the European Society of Medical Oncology (ESMO) guideline [11]. Low risk was defined as endometrioid endometrial cancers grade 1–2 with <50% myometrial invasion (MI) assessed by MRI, intermediate risk as endometrioid grade 1–2 with MI > 50%, or grade 3 endometrioid with MI <50%. Endometrioid grade 3 tumors with MI > 50% and all non-endometrioid cancers were classified as high risk. The evaluation of myometrial invasion was non-systematically performed by CT or ultrasound prior to the implementation of MRI in 2009, after which all patients were systematically grouped. Pelvic lymphadenectomy was gradually restricted (2010−2012) to the intermediate-risk group while pelvic and para-aortic lymphadenectomy was performed for the high-risk group. In 2011, PET/CT was introduced for preoperative evaluation. Any patient with PET-positive pelvic or para-aortic lymph nodes underwent lymphadenectomy, unless intolerable or complete debulking was deemed unattainable. In October 2015, preoperative immunohistochemical expression of PR and ER was included as part of a phase 4 implementation study (MoMaTEC2); in low- and intermediate-risk cases, lymphadenectomy was omitted when ER and PR expression was positive. In addition to preoperative assessment, perioperative findings (e.g. enlarged lymph nodes) could prompt lymphadenectomy. Supplementary Table 1 shows the current algorithm for extent of surgery.

2.5. Adjuvant treatment

Patients were postoperatively reclassified based on histopathological examination of the hysterectomy specimen and final FIGO stage into low-, intermediate-, or high-risk groups (endometrioid grade 3 stage IB, any stage II-IV tumors and any non-endometrioid tumors), in line with the ESMO classification [11,12]. At Haukeland University Hospital, lymphovascular space invasion status was added to the pathology report in 2018, but did not affect treatment, and is not included in our analyses. Standard adjuvant treatment in 2001–2009 was adjuvant radiotherapy (external beam +/− brachytherapy) or platinum based adjuvant chemotherapy (standard being carboplatin plus paclitaxel for six cycles) for high-risk tumors. The contemporary guidelines contained no specification for choice of modality, except for a preference for chemotherapy in serous or clear-cell tumors. From 2009, national guidelines no longer recommended adjuvant radiotherapy except for stage II patients with incomplete surgical margins. Instead, adjuvant chemotherapy was advocated for all high-risk tumors, with six cycles of carboplatin plus paclitaxel as standard treatment.

2.6. Statistical analysis

All statistical analyses were performed in SPSS 25.0 (IBM, New York) or R v3.6.1 (R Core Team 2019). Year-to-year time trends were assessed by linear regression for continuous variables and the Chi-square test for trend for proportions. Categorical variables were compared by Chi-square test or Fischer's exact test, and differences in distributions of continuous variables were assessed by the Mann-Whitney *U* test. To explore the influence of clinicopathologic variables over the observation period, a multivariable cox regression survival model was built using enter method. Age, BMI, parity, MI, histological type and grade, FIGO stage, year of treatment and adjuvant treatment modalities were analyzed in univariable analysis. Variables with hazard ratios with $p < 0.1$ were included in the adjusted multivariable analysis.

To compare different adjuvant treatment strategies the cohort was divided at 01 Jan 2010, based on the time point for national guideline change in 2009. For analysis of outcomes related to the systematic reduction in the rate of patients undergoing lymphadenectomy, 01 Jan 2013 was chosen, based on the time point where patient surgical files started containing explicit rationale for performing lymphadenectomy (gradual increase over 2010–2012).

Overall survival (OS) was defined as time from treatment to death from any cause. Disease specific survival (DSS) was defined as time from treatment to death from endometrial cancer. Recurrence-free Survival (RFS) was defined as time from surgery to first verified recurrence, and only included patients with completely resected tumors (macroscopically tumor-free). To account for differences in follow-up times due to sampling groups from different time periods, OS and DSS were reported at 5 years after primary treatment longer follow-up was blinded. RFS was analyzed at 3 years and follow-up was blinded at 3 years, as more than 70% of recurrences occur within 3 years, allowing earlier reliable assessment of RFS than OS and DSS [13]. The Kaplan-Meier method was used to visualize differences in survival between groups, using the log-rank test for comparisons between groups. For all statistical analyses, differences were considered significant at $p < 0.05$ (two-sided).

3. Results

3.1. Increasing age, BMI and serous histology over time

A total of 1308 patients were included in the study (Table 1), with a median follow-up time of 49 months (range 0–212). The number of treated patients showed an increasing trend over 2001–2019, mirroring the Norwegian increase in endometrial cancer incidence (Fig. 1A, Supplementary Table 2). Median age at primary treatment was 66 years (interquartile range 15), with an average 2 months/year increase ($p = 0.008$, Fig. 1B). Median BMI was 27.3 kg/m^2 (interquartile range 8), also with a slightly increasing trend over time (0.08 kg/m^2/year, $p = 0.037$, Fig. 1C). The distribution of FIGO stages showed some year-to-year variation, but no time-dependent trend was observed (Fig. 1D). The proportion of endometrioid endometrial cancer at postoperative histopathological diagnosis was stable, as well as histological grade within the endometrioid subtype (Fig. 1E and F). Distribution of non-endometrioid histological types was constant, apart from a statistically significant increasing trend in the proportion of serous endometrial cancer ($p = 0.004$, Fig. 1E). The proportion of serous tumors in 2010–2019 was 13%, compared to 9% in 2001–2009 (Fig. 1F).

In a Cox regression model (Supplementary Table 3), increasing age, stage III-IV, high grade EEC, NEEC, and deep myometrial invasion were all significant predictors of poor survival in both unadjusted analysis and after adjusting for all other variables ($p = 0.031$ for grade 3 EEC, $p < 0.001$ for the rest,). Year of primary treatment did not affect survival outcome. Any adjuvant treatment was associated with higher hazard ratio (for disease specific death) compared to no adjuvant treatment, however when adjusting for the other variables, radiotherapy remained significant with an adjusted hazard ratio of 1.9 (95% CI 1.1–3.4, $p = 0.035$), whereas chemotherapy did not (1.2, 95% CI 0.8–1.9, $p = 0.47$).

Table 1
Clinical and pathological characteristics of the cohort (n = 1308).

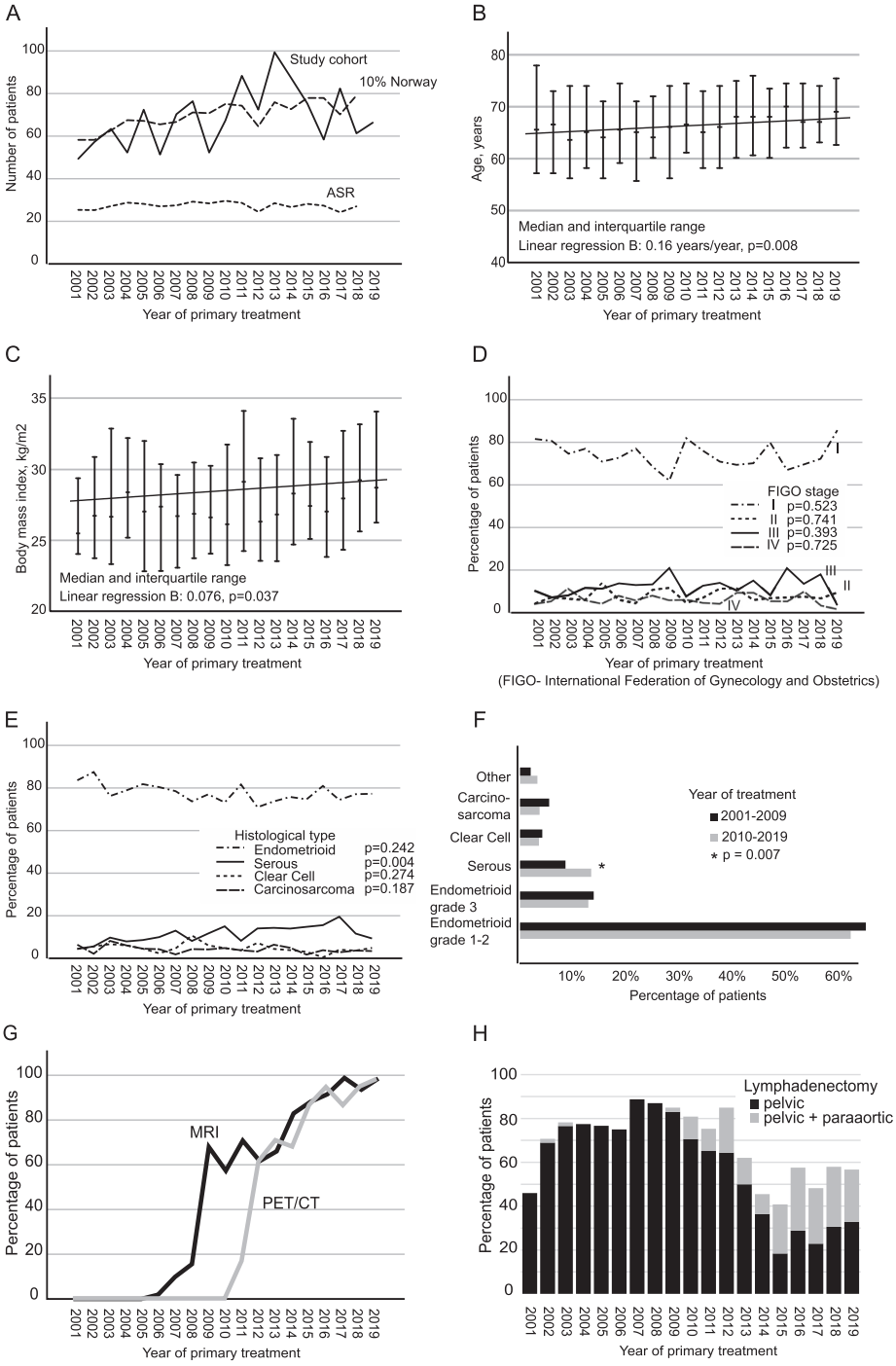
	Median	Interquartile range
Age at treatment	66	15
Body mass index	27.3	8
	n	%
Menopausal status		
Pre−/perimenopausal	130	9.9%
Postmenopausal	1177	90.1%
Parity		
Para 0	208	16.1%
Para 1+	1086	83.9%
Primary treatment		
Hysterectomy	1241	94.9%
Tumor reduction	8	0.6%
Curettage	59	4.5%
Mode of surgery (hysterectomy)		
Laparotomy	972	78.3%
Laparoscopy	92	7.4%
Robot-assisted laparoscopy	177	14.3%
Lymph node sampling		
Not performed	422	32.3%
Pelvic	742	56.7%
Para-aortic and pelvic	144	11.0%
Lymph node metastasis		
Negative	773	87.2%
Positive	113	12.8%
FIGO stage		
I	968	74.0%
II	101	7.7%
III	157	12.0%
IV	82	6.3%
Histological subtype		
Endometrioid (EEC)	1016	77.7%
Non-endometrioid	292	22.3%
Clear cell	50	3.8%
Serous papillary	148	11.3%
Carcinosarcoma	58	4.4%
Undifferentiated/other	36	2.8%
Histological Grade (EEC only)		
Grade 1–2	826	82.8%
Grade 3	172	17.2%
Adjuvant treatment		
None	863	66.0%
External radiation	81	6.2%
Brachytherapy	7	0.5%
Chemotherapy	325	24.8%
Chemotherapy + radiation	10	0.8%
Hormonal treatment	22	1.7%

FIGO International Federation of Gynecology and Obstetrics.

3.2. Reduction of lymphadenectomy with maintained rate of stage IIIC patients

MRI and PET/CT were implemented in diagnostics during the study period (Fig. 1G), peaking in 2015–2019 with >85% of patients subjected to both examinations. The rate of lymphadenectomy decreased, with a pronounced decline in 2012–2013, and flattening out to 50–60% onwards (Fig. 1H). Thus, a significantly smaller proportion of patients underwent lymphadenectomy in 2013–2019 compared to 2001–2012 (53% versus 78%, $p < 0.001$). The rate of para-aortic procedures increased (5% to 20% of all patients, $p < 0.001$) (Table 2). The rate of patients with verified lymph node metastases was stable across the two time periods (8% vs 9%, $p = 0.576$), including para-aortic metastases (Stage IIIC2; 2% vs 2%).

The group of patients not undergoing lymphadenectomy in 2013–2019 largely consisted of low- and intermediate-risk patients (based on preoperative histology and MI) without additional risk


A
Number of patients
Study cohort
10% Norway
ASR
Year of primary treatment
B
Age, years
Median and interquartile range
Linear regression B: 0.16 years/year, p=0.008
Year of primary treatment
C
Body mass index, kg/m2
Median and interquartile range
Linear regression B: 0.076, p=0.037
Year of primary treatment
D
Percentage of patients
FIGO stage
I p=0.523
II p=0.741
III p=0.393
IV p=0.725
Year of primary treatment
(FIGO- International Federation of Gynecology and Obstetrics)
E
Percentage of patients
Histological type
Endometrioid p=0.242
Serous p=0.004
Clear Cell p=0.274
Carcinosarcoma p=0.187
Year of primary treatment
F
Other
Carcino-sarcoma
Clear Cell
Serous
Endometrioid grade 3
Endometrioid grade 1-2
Year of treatment
2001-2009
2010-2019
* p = 0.007
Percentage of patients
G
Percentage of patients
MRI
PET/CT
Year of primary treatment
H
Percentage of patients
Lymphadenectomy
pelvic
pelvic + paraaortic
Year of primary treatment

factors (lymphadenopathy on imaging, loss of ER/PR or clinical upstaging) (Fig. 2A). Interestingly, among patients undergoing lymphadenectomy in this period, no patients had verified lymph node metastases in the low- and intermediate-risk groups unless having additional risk factors. Among non-lymphadenectomized low-risk patients, five out of 79 (6%) experienced recurrence within 3 years, compared to one of 25 (4%) of patients undergoing lymphadenectomy in spite of not having any apparent risk factors. Corresponding percentages for intermediate-risk patients without additional risk factors were four recurrences in 41 node negative patients (10%) and three recurrences in 43 non-lymphadenectomized patients (7%).

Survival data was available for 778 patients treated between 2001 and 2012 with a median follow-up of 71 months (range 0–212) and for 530 patients treated between 2013 and 2019 in with a median follow-up of 25 months (range 0–70). Although the proportion of stage I patients not undergoing lymphadenectomy increased from 17% to 51%, 3-year RFS was maintained in this group (0.91 (95% CI 0.86–0.96) for 2013–2019 compared to 0.88 (95% CI 0.82–0.95), $p = 0.46$, Fig. 2B). For the whole cohort comparing 2001–2012 to 2013–2019, 3-year RFS was 0.84 (95% CI 0.81–0.87) vs 0.85 (95% CI 0.81–0.89, $p = 0.56$).

3.3. Changes in adjuvant treatment with discontinuation of radiotherapy

Administration of adjuvant radiotherapy was reduced from 12% of all patients in 2001–2009 to 1% in 2010–2019 ($p < 0.001$, Fig. 3A), while the proportion of patients receiving adjuvant chemotherapy increased from 10% to 31% ($p < 0.001$). In stage I high-risk patients, 79% received chemotherapy in 2010–2019 compared to 28% in 2001–2009 ($p < 0.001$), representing the main contribution to the overall increase in use of adjuvant therapy (Fig. 3B). For (postoperative) low- and intermediate-risk patients in stage I, adjuvant therapy rates were low and stable. In stage II patients, the reduction in radiotherapy was comparable to the increase in chemotherapy, thus with a stable overall rate of adjuvant treatment in this group (62% to 58%, $p = 0.7$). The proportion of patients with stage III (macroscopically tumor-free) receiving adjuvant chemotherapy increased significantly from 27% to 95% ($p < 0.001$). The proportion not receiving any adjuvant treatment in this group decreased from 16% to 5% ($p < 0.05$).

The median follow-up time was 73 months (range 0–212) for the 2001–2009 group and 35 months (range 0–95) for the 2010–2019 group. No differences in 5-year OS or DSS between groups were found, nor for 3-year RFS (Fig. 4A). In subgroup analysis, 5-year OS improved significantly in completely resected stage III patients from 0.49 (95% CI: 0.37–0.65) to 0.61 (0.45–0.83, $p = 0.04$, Fig. 4B). RFS at 3 years in stage III was also significantly better in 2010–2019 (0.71 (95% CI: 0.39–0.68)) compared to the 2001–2009 group (0.51 (0.39–0.68, $p = 0.03$)). OS, DSS and RFS in stage I and II were similar before and after 2009. Outcome was also stable for stage I high-risk patients in spite of a substantial increase in adjuvant chemotherapy in this group (Supplementary Fig. 1). The 3-year recurrence rate for all patients for the whole observation period was 17%, with distant recurrences in 9%, pelvic in 2% and vaginal recurrences in 6% (Fig. 4C). In completely resected stage III patients the rate of distant recurrences decreased from 38% in 2001–2009 to 28% in 2010–2019, vaginal recurrences from 9% to 3% and pelvic recurrences increased from 5% to 8%, but the changes were not statistically significant.

Table 2
Comparison of extent of disease and extent and outcome of lymphadenectomy before and after 1 Jan 2013.

	2001–2012 (n = 778) n (%)	2013–2019 (n = 530) n (%)	*p* (chi-square)
FIGO Stage			0.899
I	581 (75)	384 (73)	
II	60 (8)	41 (8)	
III	91 (12)	66 (13)	
IIIc1	49 (6)	30 (6)	
IIIc2	14 (2)	9 (2)	
IV	46 (6)	35 (7)	
Lymphadenectomy (LA)			**<0.001**
Not performed	171 (22)	251 (47)	
Pelvic	567 (73)	175 (33)	
Para-aortic and pelvic	40 (5)	104 (20)	
Lymph node metastasis			0.576
Negative + unknown	708 (91)	487 (92)	
Positive	70 (9)	43 (8)	

FIGO: International Federation of Obstetrics and Gynecology.
Numbers in bold signify p-values < 0.05.

4. Discussion

Major research efforts are being deployed into uncovering the optimal ways to stage and treat endometrial cancer. Main points of controversy are the role of lymphadenectomy and matching optimal adjuvant therapy regimes to subgroups. We have performed a broad analysis in a population based Norwegian cohort to retrospectively assess the effects of national and local changes to optimize the rate of patients undergoing lymphadenectomy on one hand, and the discontinuation of adjuvant radiotherapy on the other. We describe a successful reduction of the rate of endometrial cancer patients undergoing lymphadenectomy, with maintained identification rates of stage IIIC patients and consistent low recurrence rates in unstaged patients. In addition, we have analyzed outcome after discontinuing radiotherapy as an adjuvant option and implementing adjuvant chemotherapy alone as standard treatment in high-risk patients, and find maintained overall survival outcome and improved survival in stage III patients.

Sentinel node (SN) mapping is on the rise in endometrial cancer, due to high sensitivity and negative predictive value [14]. Nevertheless, in a recent survey, 50% of gynecological oncologists in Europe and USA did not use this technique, implying that for many institutions a better risk-stratification of patients prior to surgical staging is still an important issue [15]. At our institution, where sentinel node mapping is not implemented, the rate of patients undergoing lymphadenectomy has decreased over the last 6–7 years. This is due to a shift from universal sampling to selective lymphadenectomy, following an incorporation of imaging and molecular biomarkers into the diagnostic work-up. We show that in spite of a marked reduction in lymphadenectomies, we still identify metastatic nodes at the same rate, and there is no indication of increased recurrence rates in the non-staged patients. For institutions using sentinel node techniques, these results may also be of interest, especially when failed mapping mandates a full- or hemipelvic lymphadenectomy [16]. Even when successful, sentinel node procedures add significant time and cost to surgery compared to no lymph node removal, and should be omitted when unnecessary [17].

Fig. 1. Time trends in clinicopathological characteristics 2001–2019. A) Number of endometrial cancer patients receiving primary treatment at Haukeland University Hospital. The stippled lines show the incidence in Norway divided by 10 and the Norwegian age-standardized rate per 100,000 person years (ASR) based on 2014 age weights [2]. Full numerical data in Supplementary Table 1. B) Age at primary treatment, median and inter-quartile range with linear regression $y = Bx + k$. C) Body mass index, median and inter-quartile range with linear regression $y = Bx + k$. D) Trend in distribution of FIGO stages. Trends analyzed with chi-square test for trend. E) Trend in distribution of histopathological subtypes in final surgical specimen. Trends analyzed with chi-square test for trend. F) Distribution of histologic types in final surgical specimen split by decade. Other includes undifferentiated and rare histological subtypes. G) Changes in the use of magnetic resonance imaging (MRI) and positron emission tomography/computerized tomography (PET/CT). MRI was included in routine management from 2009, PET/CT from 2011. H) Changes in rates of lymphadenectomy, and extent of procedure.

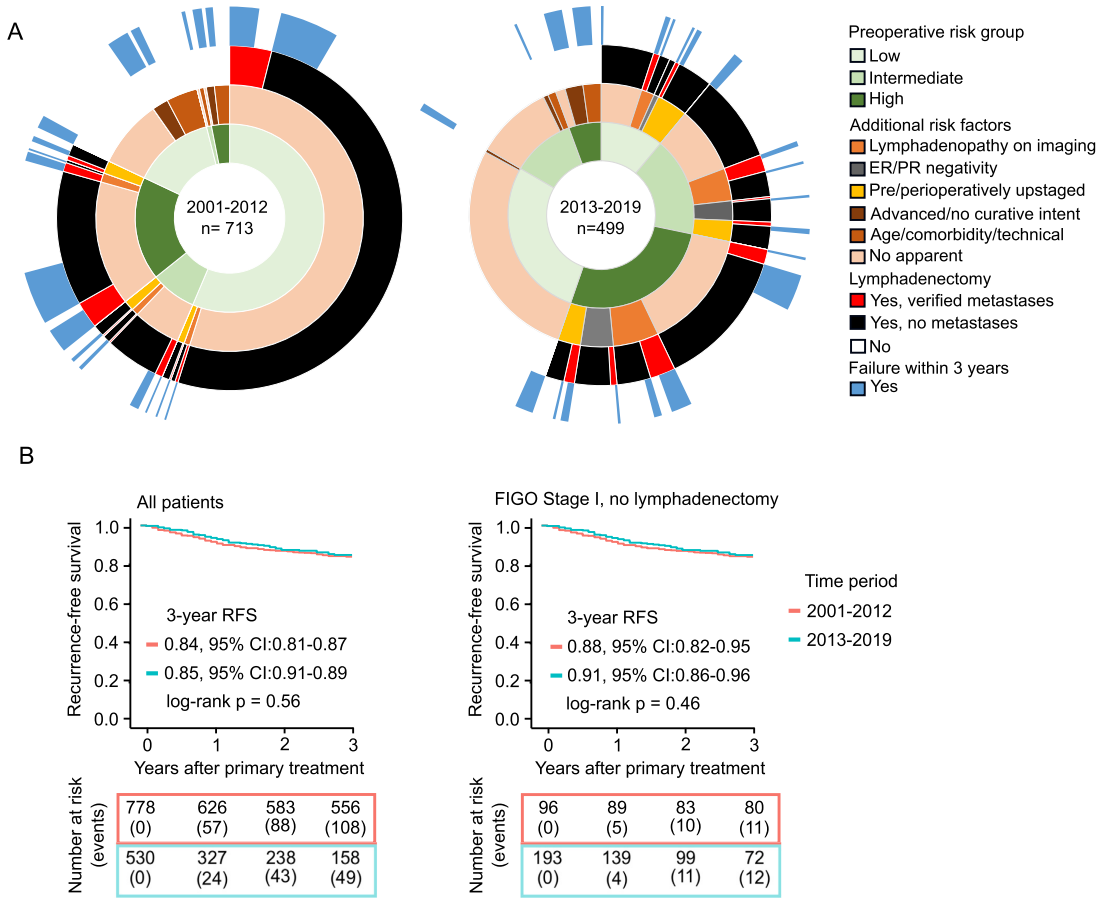


Fig. 2. A) Preoperative characterization of hysterectomized patients, before and after 01 Jan 2013, with a preoperative endometrial cancer assessment (excluding incidental findings after benign diagnosis and surgery for presumed ovarian cancer). Inner circle displays risk groups based on histologic assessment of preoperative biopsy/curettage and imaging: Low: endometrioid grade 1–2 with <50% myometrial invasion (MI) or MI unknown. Intermediate: endometrioid grade 1–2 with >50% MI or grade 3 with <50% MI. High: endometrioid grade 3 with >50% MI or MI unknown and all non-endometrioid cancers. Patients missing preoperative histological info were excluded (n = 31). Second circle displays the additional risk factor most important for explaining whether patients underwent LA, based on patient file review. Third circle displays prevalence of metastatic lymph nodes where LA was performed. Outer circle displays recurrences or progression occurring within 3 years. All sectors correspond to proportions of patients included. ER Estrogen Receptor, PR Progesterone Receptor, pre/perioperatively upstaged signifies imaging or clinical findings corresponding to stage>I (other than lymphadenopathy), technical signifies perioperative technical issues due to adhesions, bleeding, also including patient's wish. B) Kaplan-Meier survival curve showing 3-year recurrence free survival before and after reduction of lymphadenectomies in 2013. FIGO: International Federation of Gynecology and Obstetrics.

We report an increase in use of adjuvant chemotherapy in high-risk patients (stage I high-risk + stage II and III), and a concomitant cessation of adjuvant radiotherapy. Although adjuvant therapy for high-risk patients is in line with current international recommendations, the optimal treatment algorithm is under debate, especially concerning the respective roles of radiotherapy and systemic chemotherapy. The ESMO consensus favors external beam radiation therapy for stage I high-risk patients when staged and node negative, and supports consideration of brachytherapy, but states that the role of systemic chemotherapy is insufficiently investigated [12]. In trials with stage I high-risk patients where chemotherapy alone has been compared with radiotherapy, no differences in OS or RFS have been shown, although pelvic recurrence rates were lower after radiotherapy and distant recurrences lower after chemotherapy [18,19]. Our study shows that omitting radiotherapy in stage I patients has not produced poorer outcome, when substituted with chemotherapy. Advantages with this approach is avoidance of radiotherapy related side effects and saving radiotherapy for salvage treatment of vaginal and small pelvic recurrences in patients if they do occur. We report a vaginal recurrence rate of 6% in the whole population-based series, which seems comparable to 5–10% in previously published chemotherapy only-studies reporting high-risk cases [18–20]. We do note that the substantial increase in adjuvant chemotherapy in stage I patients does not seem to improve outcome. The ongoing ENGOT-EN2-DGCG/EORTC55102 study (clingov ID NCT01244789), comparing adjuvant chemotherapy with observation for low-stage high-risk patients will hopefully provide additional data to optimize treatment strategies for this group. Molecular subtyping provides prognostic information independent of classical histopathological stratification and could improve tailoring of treatment [21].

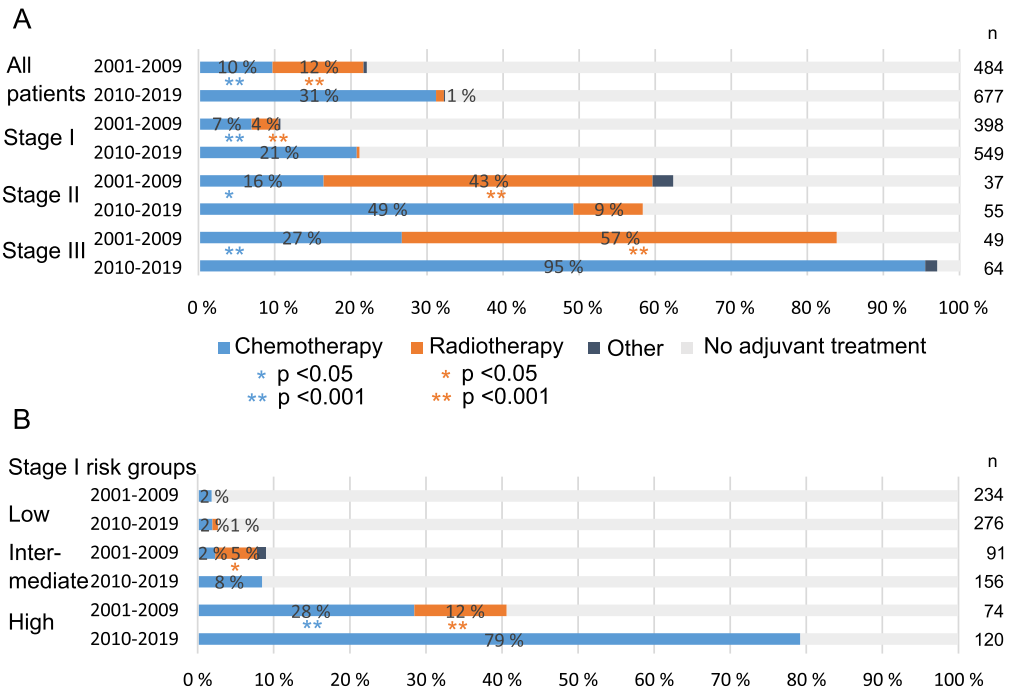


Fig. 3. A) Changes in administration of adjuvant treatment between 2001 and 2009 and 2010–2019. Hysterectomized patients with macroscopically resectable tumors included. Other includes hormonal treatment (n = 5), brachytherapy alone (n = 2) and chemoradiation (n = 1). Statistical comparison between use of chemotherapy/radiotherapy in the different time periods by Chi-square or Fischer's exact test (2-sided) where appropriate. B) Stage I risk groups based on histologic assessment of preoperative biopsy/curettage: low; endometrioid grade 1–2 with <50% myometrial invasion (MI) or MI unknown, intermediate; endometrioid grade 1–2 with >50% MI on imaging or grade 3 with <50% MI, and high; endometrioid grade 3 with >50% MI or MI unknown and all non-endometrioid cancers. FIGO: International Federation of Gynecology and Obstetrics.

As of yet, no published prospective data regarding management of endometrial cancer by molecular subtype is available.

Unlike early stage endometrial cancer, for advanced endometrial cancer patients there is strong evidence in favor of adjuvant chemotherapy. In the GOG-122 trial, chemotherapy demonstrated superior OS and progression-free survival to radiotherapy for stage III-IV patients, and was non-inferior to the combination of chemotherapy and radiotherapy in GOG-258, although the pelvic recurrence rate was higher for chemotherapy alone [7,22]. In the present study, improvement in OS and RFS for stage III patients was observed, coinciding with an overall increase in adjuvant treatment, and at the same time a cessation of radiotherapy. Similar survival and recurrence rates have been demonstrated in a separate Norwegian high-risk cohort [20]. The low rate of vaginal recurrences in stage III patients is interesting. However, a low number of stage III patients could affect this result, and the drop from 9% to 3% was not statistically significant. Preoperative MRI and PET/CT could increase the proportion of stage IIIC patients with limited uterine disease, and thus lower the risk for local recurrence as observed in our study, but this needs to be confirmed in future studies. The PORTEC-3 trial recently demonstrated improved OS and failure-free survival when combining chemotherapy and radiotherapy compared to radiotherapy alone in high-risk patients, mainly driven by improved results in stage III patients and with an increased rate of adverse events and persisting morbidity [23]. Adjuvant chemotherapy alone was not explored in PORTEC-3, thus the available evidence today does not support a benefit of adding radiotherapy when adjuvant chemotherapy constitutes the management strategy for advanced stage endometrial cancer, again reflected in the analysis of the present population based series.

Our retrospective study is limited in its inability to establish clear cause-effect relationships, especially in evaluating contributions of different diagnostic methods towards a reduction of the overall rate of patients undergoing lymphadenectomy. We are however, at this time satisfied to point out that the rate of lymphadenectomy can be reduced, and that in our setting, no apparent detrimental effect is seen. Preoperative risk grouping to tailor surgery depends on a high concordance between the diagnostic workup and final diagnosis. We have previously shown that there is histological discordance between biopsy and hysterectomy specimen in 16%, and that the MRI diagnosis of cervical invasion and deep myometrial invasion have an accuracy of 79%–89% and 61–68% respectively, and thus additional parameters are necessary to optimize a selective lymphadenectomy algorithm [24,25]. Availability of imaging modalities including MRI and PET differs between institutions and they are not standard of care in many countries. Immunohistochemical analysis of ER and PR however, carries little extra cost and is potentially beneficial for clinics without access to advanced imaging. Improvement of the selective lymphadenectomy algorithm with focus on cost effectiveness is an important aim for future research.

Another potential bias is the shorter follow-up time for the patients treated in the most recent time period. We have attempted to compensate for this by choosing appropriate outcome for comparison. This is especially relevant for lymphadenectomy frequencies, where the most recent group has a median follow-up time of 25 months. Data maturation will enable a better estimate of the recurrence rate and survival of low-stage patients not undergoing lymphadenectomy, and will be reported when finalizing the MoMaTEC2 study. We were unable to retrospectively quantify treatment related complications in our study, as

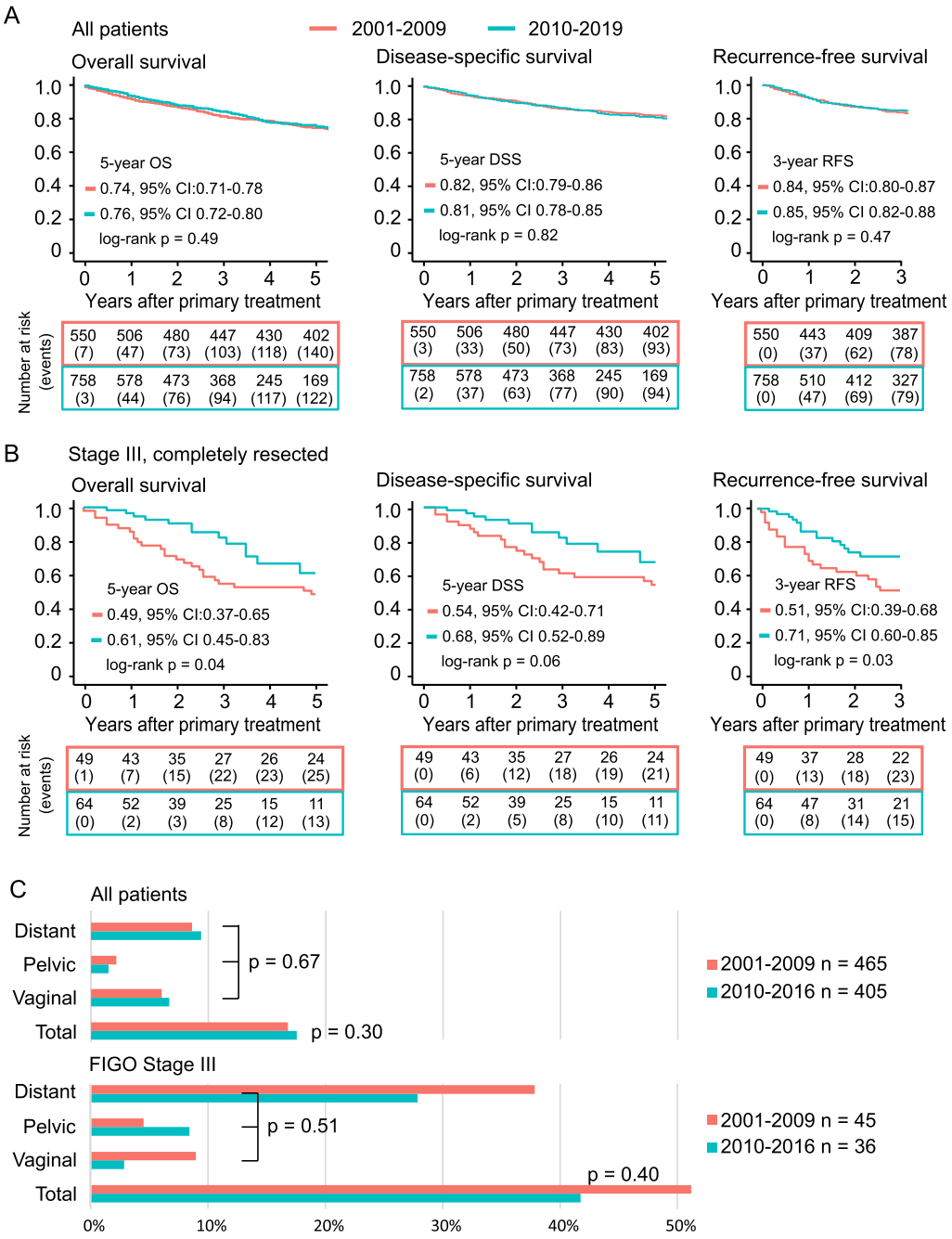


Fig. 4. Kaplan-Meier survival curves showing survival outcome before and after omitting radiotherapy as adjuvant treatment for A) all patients. B) completely resected FIGO stage III patients. C) Recurrence rate by site at 3 years in completely resected patients. Patients censored before 3 years not included. Statistical comparison of groups with chi-square. FIGO: International Federation of Gynecology and Obstetrics.

these have not been systematically registered clinically. There is a need for prospective data on patient reported outcomes for different treatment modalities, to better understand tolerability in short and long term.

In conclusion, we present data from a population based endometrial cancer cohort over the span of two decades, and show that changing to a strategy of individualized risk-based stratification for lymphadenectomy does not affect survival outcomes negatively, when compared to the previous practice based on more frequent lymphadenectomy. Additionally, our data supports that adjuvant treatment without radiotherapy is feasible with maintained survival and was even associated to improved survival for stage III patients.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2020.12.002.

Declaration of competing interest

There are no conflicts of interest to disclose.

Acknowledgement

We thank Ellen Valen, Britt Edvardsen, Kadri Madisoo, and Elisabeth Enge for technical assistance and Kristina Lindemann for valuable input.

This study was supported by Helse Vest (HV440088), the University of Bergen, the Norwegian Cancer Society (190202-2017), the Research Council of Norway, and Bergen Research Foundation.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 68 (2018) 394–424.

[2] Norwegian Cancer Registry, https://sb.kreftregisteret.no/insidens 2018 Date of Access: 09 JAN 2020.

[3] P. Morice, A. Leary, C. Creutzberg, N. Abu-Rustum, E. Darai, Endometrial cancer, Lancet (London, England) 387 (2016) 1094–1108.

[4] J.A. How, P. O'Farrell, Z. Amajoud, S. Lau, S. Salvador, E. How, et al., Sentinel lymph node mapping in endometrial cancer: a systematic review and meta-analysis, Minerva Ginecol. 70 (2018) 194–214.

[5] C.L. Creutzberg, K.H. Lu, G.F. Fleming, Uterine cancer: adjuvant therapy and management of metastatic disease, J. Clin. Oncol. 37 (2019) 2490–2500.

[6] B. Hagen, Veileder i gynekologisk onkologi 2009, https://www.legeforeningen.no/contentassets/04d0b3c134ac4b12aa1a03c3a2666585/veileder-i-gynekologisk-onkologi-2009.pdf Date of Access: 11 DEC 2019.

[7] M.E. Randall, V.L. Filiaci, H. Muss, N.M. Spirtos, R.S. Mannel, J. Fowler, et al., Randomized phase III trial of whole-abdominal irradiation versus doxorubicin and cisplatin chemotherapy in advanced endometrial carcinoma: a gynecologic oncology group study, J. Clin. Oncol. 24 (2006) 36–44.

[8] Statistics Norway, https://www.ssb.no/befolkning 2020 Date of Access: 11 MAR 2020.

[9] H.M. Werner, J. Trovik, J. Marcickiewicz, S. Tingulstad, A.C. Staff, F. Amant, et al., Revision of FIGO surgical staging in 2009 for endometrial cancer validates to improve risk stratification, Gynecol. Oncol. 125 (2012) 103–108.

[10] K. Kinkel, R. Forstner, F.M. Danza, L. Oleaga, T.M. Cunha, A. Bergman, et al., Staging of endometrial cancer with MRI: guidelines of the European Society of Urogenital Imaging, Eur. Radiol. 19 (2009) 1565–1574.

[11] N. Colombo, E. Preti, F. Landoni, S. Carinelli, A. Colombo, C. Marini, et al., Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 24 (2013) vi33-vi8.

[12] A. Casado, A. González-Martín, A. Rodolakis, A. Taylor, A. Westermann, A.G. Zeimet, et al., ESMO-ESGO-ESTRO consensus conference on endometrial cancer: diagnosis, treatment and follow-up, Ann. Oncol. 27 (2015) 16–41.

[13] I. Vistad, L. Bjørge, O. Solheim, B. Fiane, K. Sachse, J. Tjugum, et al., A national, prospective observational study of first recurrence after primary treatment for gynecological cancer in Norway, Acta Obstet. Gynecol. Scand. 96 (2017) 1162–1169.

[14] E.C. Rossi, L.D. Kowalski, J. Scalici, L. Cantrell, K. Schuler, R.K. Hanna, et al., A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): a multicentre, prospective, cohort study, Lancet Oncol. 18 (2017) 384–392.

[15] J. Casarin, F. Multinu, N. Abu-Rustum, D. Cibula, W.A. Cliby, F. Ghezzi, et al., Factors influencing the adoption of the sentinel lymph node technique for endometrial cancer staging: an international survey of gynecologic oncologists, Int. J. Gynecol. Cancer 29 (2019) 60.

[16] R.W. Holloway, N.R. Abu-Rustum, F.J. Backes, J.F. Boggess, W.H. Gotlieb, W. Jeffrey Lowery, et al., Sentinel lymph node mapping and staging in endometrial cancer: a Society of Gynecologic Oncology literature review with consensus recommendations, Gynecol. Oncol. 146 (2017) 405–415.

[17] B. Geppert, C. Lonnerfors, M. Bollino, J. Persson, Sentinel lymph node biopsy in endometrial cancer-Feasibility, safety and lymphatic complications, Gynecol. Oncol. 148 (2018) 491–498.

[18] N. Susumu, S. Sagae, Y. Udagawa, K. Niwa, H. Kuramoto, S. Satoh, et al., Randomized phase III trial of pelvic radiotherapy versus cisplatin-based combined chemotherapy in patients with intermediate- and high-risk endometrial cancer: a Japanese Gynecologic Oncology Group study, Gynecol. Oncol. 108 (2008) 226–233.

[19] R. Maggi, A. Lissoni, F. Spina, M. Melpignano, P. Zola, G. Favalli, et al., Adjuvant chemotherapy vs radiotherapy in high-risk endometrial carcinoma: results of a randomised trial, Br. J. Cancer 95 (2006) 266–271.

[20] E. Smogeli, M. Cvancarova, Y. Wang, B. Davidson, G. Kristensen, K. Lindemann, Clinical outcome of patients with high-risk endometrial carcinoma after treatment with chemotherapy only, Int. J. Gynecol. Cancer 28 (2018) 1789–1795.

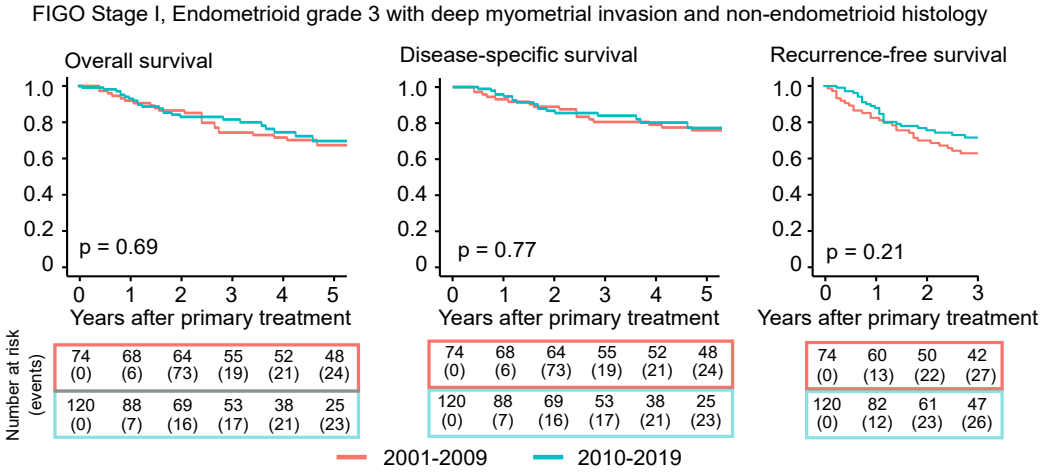
[21] C. Kandoth, N. Schultz, A.D. Cherniack, R. Akbani, Y. Liu, H. Shen, et al., Integrated genomic characterization of endometrial carcinoma, Nature. 497 (2013) 67–73.

[22] D. Matei, V. Filiaci, M.E. Randall, D. Mutch, M.M. Steinhoff, P.A. DiSilvestro, et al., Adjuvant chemotherapy plus radiation for locally advanced endometrial cancer, N. Engl. J. Med. 380 (2019) 2317–2326.

[23] S.M. de Boer, M.E. Powell, L. Mileshkin, D. Katsaros, P. Bessette, C. Haie-Meder, et al., Adjuvant chemoradiotherapy versus radiotherapy alone in women with high-risk endometrial cancer (PORTEC-3): patterns of recurrence and post-hoc survival analysis of a randomised phase 3 trial, Lancet Oncol. 20 (2019) 1273–1285.

[24] H.M.J. Werner, J. Trovik, J. Marcickiewicz, S. Tingulstad, A.C. Staff, M.E. Engh, et al., A discordant histological risk classification in preoperative and operative biopsy in endometrial cancer is reflected in metastatic risk and prognosis, Eur. J. Cancer 49 (2013) 625–632.

[25] I.S. Haldorsen, J.A. Husby, H.M. Werner, I.J. Magnussen, J. Rørvik, H. Helland, et al., Standard 1.5-T MRI of endometrial carcinomas: modest agreement between radiologists, Eur. Radiol. 22 (2012) 1601–1611.



Supplementary Fig. 1. Kaplan–Meier curves for FIGO stage I high-risk patients.
FIGO: International Federation of Gynecology and Obstetrics.

Supplementary table 1. Treatment algorithm at Haukeland University Hospital (2019)

Presumed FIGO Stage I	**Type**	**Grade**	**MI**	**SIZE>5cm**	**ER/PR**	**LNM**	**Treatment**
Low risk	EEC	1-2	<50%	-	-	-	Hysterectomy
				+			+ pelvic LA
					+		+ pelvic LA
						+	+ pelvic (±para-aortic LA)
Intermediate risk	EEC	1-2	≥50%	-	-	-	Hysterectomy
	EEC	3	<50%	-	-	-	
				+			+ pelvic LA
					+		+ pelvic LA
						+	+ pelvic (±para-aortic LA)
High risk	EEC	3	≥50%	+/-	+/-	+/-	Hysterectomy + pelvic and para-aortic LA
	NEEC		any	+/-	+/-	+/-	Hysterectomy + pelvic and para-aortic LA + omentectomy
FIGO Stage II-III							
Cervical stromal infiltration (FIGO Stage II)							Wertheim-Meigs (radical) hysterectomy ± pelvic and para-aortic LA
Resectable Stage III							Hysterectomy + pelvic and para-aortic LA
Palliative intent (advanced cancer or patient issues)							Hysterectomy/tumor reduction/non-surgical treatment

FIGO International federation of gynecology and obstetrics, ER/PR loss of expression of estrogen/progesterone receptor in curettage/biopsy (<30% nuclei positive for either ER or PR), MI Myometrial infiltration (CT/MRI), SIZE maximum tumor diameter (imaging), LNM lymphadenopathy on CT, MRI or PET/CT, EEC Endometrioid endometrial cancer, NEEC non-endometrioid endometrial cancer, LA lymphadenectomy

Supplementary table 2. The study cohort compared to endometrial cancer statistics drawn from the Norwegian Cancer Registry.

	Norway			Hordaland (primary uptake area)			Study cohort			
Year	Cases	Rate[1]	ASR[2]	Cases	Rate[1]	ASR[2]	Total	Local	Referred	%
2001	589	25.9	26.0	62	28.1	29.5	50	47	3	6 %
2002	589	25.7	25.9	59	26.7	27.8	58	50	8	14 %
2003	633	27.5	27.8	78	35.0	37.9	64	57	7	11 %
2004	682	29.5	29.4	52	23.2	24.9	53	49	4	8 %
2005	678	29.1	28.8	78	34.5	35.9	73	65	8	11 %
2006	661	28.2	27.7	54	23.7	24.4	51	48	3	6 %
2007	673	28.4	28.2	65	28.2	30.4	71	62	9	13 %
2008	718	30.0	29.9	63	27.1	28.1	77	66	11	14 %
2009	714	29.5	29.1	54	22.9	23.3	53	45	8	15 %
2010	758	31.0	30.3	68	28.4	28.7	67	57	10	16 %
2011	749	30.3	29.3	86	35.5	35.4	88	73	15	17 %
2012	652	26.1	25.1	67	27.3	27.3	73	54	19	26 %
2013	766	30.3	29.2	86	34.6	34.9	99	78	21	22 %
2014	733	28.7	27.3	69	27.5	27.4	88	71	17	19 %
2015	785	30.5	28.9	61	24.0	23.9	74	53	21	29 %
2016	785	30.2	28.0	67	26.2	25.7	57	48	9	16 %
2017	708	27.0	24.9	74	28.8	27.9	81	69	12	15 %
2018	797	30.2	27.7	78	30.2	29.1	62	50	12	19 %
Average/year	704	28.8	27.9	68	28.4	29.0	69	58	18	15 %

[1] Cases/100 000 person years, source (https://sb.kreftregisteret.no/insidens/)

[2] Age standardized rates as computed by Norwegian cancer registry (weights based on 2014 age distribution)

Local; patients within primary district for Haukeland University Hospital

Referred; patients referred from neighboring counties with low risk endometrial cancer surgery.

% Referred of total in study cohort

Supplementary table 3. Survival analysis by Cox proportional hazards regression model. N = 1167, events (disease specific death<5y n = 147). Patients missing data for any variable are excluded.

Variable	n	Unadjusted HR [95% CI]		p	Adjusted HR [95% CI]		p
Year of primary treatment	1167	1.00	[0.96-1.04]	0.53			
Age[1]	1167	1.06	[1.04-1.07]	**<0.001**	1.03	[1.01-1.05]	**0.001**
BMI[1]	1167	0.97	[0.95-1]	0.056	1.01	[0.98-1.03]	0.717
Parity							
0	187	1.00					
1+	980	0.84	[0.55-1.27]	0.40			
Histologic type/grade[1]							
EEC grade 1-2	765	1.00			1.00		
EEC grade 3	156	3.73	[2.25-6.18]	**<0.001**	1.79	[1.06-3.04]	**0.031**
Non- EEC	246	9.85	[6.66-14.56]	**<0.001**	5.17	[3.26-8.21]	**<0.001**
FIGO Stage[1]							
I-II	973	1.00			1.00		
III-IV	194	10.51	[7.54-14.65]	**<0.001**	3.74	[2.49-5.62]	**<0.001**
Myometrial invasion[1]							
<50%	701	1.00			1.00		
>50%	466	5.77	[3.96-8.4]	**<0.001**	2.39	[1.55-3.69]	**<0.001**
Adjuvant treatment[1]							
None	788	1.00			1.00		
Radiotherapy	67	5.47	[3.25-9.22]	**<0.001**	1.89	[1.05-3.43]	**0.035**
Chemotherapy	295	6.19	[4.25-9.02]	**<0.001**	1.18	[0.75-1.87]	0.47
Other[2]	17	10.75	[5.05-22.89]	**<0.001**	2.73	[1.2-6.2]	**0.017**

[1] Variables included in multivariable model

[2] Including brachytherapy (n= 6), radiotherapy+chemotherapy (n=4), hormonal therapy (n=7)

HR: hazard ratio, CI: confidence interval
EEC: Endometrioid endometrial cancer, FIGO: International Federation of Gynecology and Obstetrics

Longitudinal effects of adjuvant chemotherapy and lymph node staging on patient-reported outcome in endometrial cancer survivors: a prospective cohort study

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ABSTRACT

Background: Most endometrial cancer patients with localized disease are effectively treated and survive for a long time. Primary treatment is hysterectomy, to which surgical staging procedures may be added to assess the need for adjuvant therapy. Longitudinal data on patient-reported outcomes comparing different levels of primary treatment is lacking, especially when adjuvant radiotherapy is omitted.

Objectives: We assessed the impact of lymphadenectomy and adjuvant chemotherapy on patient-reported symptoms, function and quality of life. We hypothesized that these treatment modalities would substantially affect patient-reported outcome at follow-up.

Study design: We prospectively included endometrial cancer patients enrolled in the ongoing MoMaTEC2 study (clingov id NCT02543710). Patients were asked to complete the patient-reported outcome questionnaires EORTC-QLQ-C30 and EORTC-EN24 preoperatively and at 1 and 2 years of follow-up. Functional domains and symptoms were analyzed for the whole cohort and by treatment received. To assess the effect of the individual treatment modifications we used mixed regression models.

Results: Of 448 included patients at baseline, 339 and 219 had reached one- and two-year follow-up. Overall, patients reported improved global health status/quality of life (+9 units, $P < 0.001$), increased emotional and social functioning and increased sexual interest and activity ($P < 0.001$ for all) from baseline to year one, and these remained stable at year two. Means of functional scales and quality of life were similar to an age- and sex-weighted reference cohort. Mean tingling/numbness and lymphedema increased after treatment. Compared to the group treated with hysterectomy and salpingo-oophorectomy only, the group who received adjuvant chemotherapy had a larger mean reduction in physical functioning (-6 versus +2, P = 0.002) at year 1, more neuropathy (+30 versus +5, $P < 0.001$, year 1) at year 1 and 2, and more lymphedema at year 1 (+11 versus +2, P = 0.007). In patients not receiving adjuvant chemotherapy, patient-reported outcomes were similar regardless of lymph node staging procedures. Adjuvant chemotherapy independently increased fatigue, lymphedema, and neuropathy in mixed regression models.

Conclusion: Endometrial cancer patients receiving adjuvant chemotherapy report significantly reduced functioning and more symptoms up to two years after treatment. For patients treated

by surgery alone, surgical staging does not appear to affect quality of life or symptoms to a measurable degree at follow-up. Subjecting patients to lymph node removal to tailor adjuvant therapy therefore seems justified from the patient's viewpoint, while efforts should increase to find alternatives to traditional chemotherapy.

INTRODUCTION

Endometrial cancer is the sixth most common cancer in women, with a lifetime risk reaching 2-3% in many industrialized countries.[1] Surgery is the cornerstone of treatment, consisting of hysterectomy and bilateral salpingo-oophorectomy, with the addition of lymph node staging (LNS) to assess the extent of spread and adjuvant radiation or chemotherapy for patients at a high risk of recurrence.[2] With an excellent 5-year survival at >90% for localized disease, treatment-related complications and post-treatment health-related quality of life (HRQL) are gaining attention. Patient-reported outcome (PRO) data regarding these issues is still scarce, but suggests benefits for minimally invasive surgery over laparotomy[3, 4], sentinel node biopsy over lymphadenectomy[5, 6], and potential long-term gastrointestinal symptoms for patients undergoing adjuvant radiotherapy[7-9]. Less is known about the effects of adjuvant chemotherapy on endometrial cancer survivors, in particular beyond the initial treatment period. Many institutions, especially in the Nordic countries, have discontinued the use of adjuvant radiotherapy in favor of chemotherapy, based on data suggesting equal or better survival[10-12], and the possibility of reserving radiotherapy for salvage treatment. PRO data for patients undergoing these types of treatment algorithms may be helpful in identifying and quantifying treatment-related problems and contribute to better information to patients and prioritization of clinical efforts and research but are not yet available.

We evaluated prospectively registered PROs in treatment groups defined by the Norwegian national guidelines for treatment of endometrial cancer, comprising selective lymphadenectomy or sentinel node biopsy and adjuvant chemotherapy for high-risk cases. We hypothesized that undergoing lymphadenectomy and/or adjuvant chemotherapy would have significant health effects that could be detected by self-reported outcome measurements.

METHODS

Ethical considerations

The study has been approved according to Norwegian legislation by the Western Regional Committee for medical and health Research Ethics (REK2015/0548). All included patients gave written informed consent.

Patient series

MoMaTEC2 is an ongoing international multicenter phase 4 study (clinicaltrials.gov ID NCT02543710), for the implementation of preoperative assessment of hormone receptors as biomarkers to guide treatment in endometrial cancer. PROs are collected as secondary endpoints. All patients treated at Norwegian participating centers undergoing hysterectomy between 15 October 2015 and 11 November 2020 were eligible for this study. Clinicopathological characteristics and treatment information were collected at baseline. Patients with advanced disease (not completely resected at primary treatment) and patients receiving adjuvant treatment other than chemotherapy or additional second-line treatment due to recurrence were excluded (Figure 1). Treatment details for included patients are listed in Table 1 and treatment principles are outlined in detail in Appendix A.

Separate consent for PRO follow-up was obtained at inclusion, with 467 patients consenting to participate (participation rate 71%). PRO respondents and non-respondents had largely similar clinical profile (Supplementary table 1).

Included patients were grouped based on treatment received: Hysterectomy and bilateral salpingo-ooforectomy (BSO) alone (Hyst group), hysterectomy with BSO and lymph node staging (LNS group), and hysterectomy and BSO with adjuvant chemotherapy, with or without LNS (Chemo group) (Figure 1).

Patient-reported outcome

The general European Organisation for Research and Treatment of Cancer – Quality of Life Questionnaire C30 (EORTC-QLQ-C30) version 3 and endometrial cancer specific EORTC-QLQ-EN24 questionnaires were completed pre-operatively (baseline) and annually post-treatment. These questionnaires are validated to describe different/complementing dimensions of function and symptoms for endometrial cancer patients and are available in Norwegian[13, 14]. Norwegian reference data from EORTC-QLQ-C30 were extracted from a previous survey in

an unselected Norwegian population and adjusted by age and gender to reflect the study cohort[15].

Function and symptom scales were derived according to the EORTC scoring manual[16] for scales that were considered relevant for our patient group. For functional scales, a positive change signifies improved function, whereas for symptomatic scales a positive change signifies increased amount of symptom, i.e., a deterioration. Response rates for most analyzed scales were found to be consistently high (97-100%) at each time point (Supplementary table 2). Exceptions were sexual interest and sexual activity with response rates of 93% and 94% at baseline.

To evaluate the clinical impact of changes for EORTC scales, Cohen's d was used to represent effect size (ES), defined as the change in means divided by the pooled standard deviation.[17] We established cutoffs for our cohort by using the standard deviation of baseline values. Changes were interpreted according to Cohens general criteria as <0.2 – trivial, 0.2-0.5 small, 0.5-0.8 moderate, >0.8 large. These values are arbitrary, however the 0.5 cutoff has been shown to be valid as a surrogate for a clinically relevant difference in HRQL assessment.[18] We compared these effect sizes to previously published anchor based cutoffs[19] and found little deviation (Supplementary table 3).

To explore the development of relevant symptoms over time, a case-wise analysis of the EORTC-QLQ-EN24 items regarding lymphedema and neuropathy (tingling/numbness) was performed in patients with completed 2 years follow-up. For this purpose, item responses were dichotomized into "no/light symptoms" ("None" or "A little") and "moderate/severe symptoms" ("Quite a bit" or "Very much"). For lymphedema, the most severe of the two corresponding item responses was selected.

Statistical Analysis

All statistical analyses were performed in R version 4.0.2 (R Core Team 2020) R Foundation for Statistical Computing, Vienna Austria).

Missing entries were analyzed for non-randomness using the R package 'Finalfit'. Imputation was performed according to the EORTC scoring manual to compute scales in spite of missing items if < 50% of relevant items were missing.[16] Missing questionnaires due to short follow-up were perceived as missing completely at random. Missing scale scores were perceived as missing at random related to treatment variables and dropped. This resulted in complete case

analysis for statistical analyses comparing year to year changes except linear mixed models which can handle missing at random data points in longitudinal analysis through maximum likelihood modelling.

Categorical variables were compared by Chi-square test or Fischer's exact test where appropriate, and differences in distributions of continuous variables were assessed by Mann-Whitney test for two groups or Kruskal-Wallis test for multiple group comparisons.

To assess changes in PRO scales over time for the entire cohort, Wilcoxon signed-rank test was used to compare changes in means from baseline to year one and two. To assess differences between treatment groups at specific time points, the Mann-Whitney test was used.

To explore how different treatment modalities independently affected PROs, effect magnitudes of EORTC scale changes were assessed, as described by the SISAQOL consortium.[20] For each scale, a linear mixed model (R packages 'lme4', 'lmerTest') was fitted with the scale score as dependent variable, a subject level random intercept, time and treatment factors as independent variables, and a baseline score covariate. Included treatment effects were surgical modality (laparoscopy or laparotomy), any LNS procedure including sentinel node biopsy and pelvic +/- paraaortic lymphadenectomy, and adjuvant chemotherapy (yes/no). Interaction terms between time and LNS and time and adjuvant chemotherapy were included to account for differences between year 1 and 2 of follow-up. In addition, separate models were explored where patients who underwent sentinel node biopsy with removal of ≤4 nodes were grouped with patients without any lymph node sampling. Effect estimates (regression coefficients) with 95% confidence intervals and p-values were reported for all mixed models. *P*-values < 0.05 were considered statistically significant in all analyses.

RESULTS

At baseline, 448 patients had consented to participate in the PRO follow-up, of which 339 and 219 patients had completed follow-up at year 1 and year 2, respectively (Figure 1). LNS had been performed in 56% of participating patients, and 32% had received adjuvant chemotherapy (Table 1). The treatment groups had similar age and body mass index distribution but differed in treatment- and histopathological characteristics (Table 1). Patients in the Chemo group more often had undergone laparotomy (69% compared to 32% in the LNS group and 9% in the Hyst group ($P < 0.001$, Table 1). Among patients in the Chemo group 39% had undergone a para-aortal dissection compared to 10% in the LNS group. Only 14% of the Chemo group had not undergone any LNS. The Chemo group had significantly higher FIGO stage and more aggressive histological subtypes ($P < 0.001$ for both). The rate of recurrences at 2 years was higher in the Chemo group (9.4% versus 4.5% and 2.8% for LNS and Hyst groups, $P = 0.039$).

Patient-reported functioning

In the overall cohort, global health status/quality of life increased from baseline to year 1 (+9 units, $P < 0.001$) and remained stable at year 2 (Table 2). Emotional function increased moderately from mean score 75 to 87 at year 1 and was stable at year 2, ($P < 0.001$). Baseline average scores for these estimates were close to or slightly below the general population reference values, whereas the higher year 1 values were slightly above reference values. Sexual functioning and sexual activity likewise increased after treatment and remained stable at year 2.

There was a small deterioration in physical functioning (-6 units at year 1 and -8 units at year 2) in the Chemo group compared to baseline, whereas changes were trivial in the other two groups (Figure 2, Supplementary table 4). Emotional function improved significantly more in the LNS group than in the Hyst group ($P = 0.005$ at year 1, $P = 0.017$ at year 2).

Patient-reported symptoms

Mean scores for lymphedema, tingling/numbness and muscular pain increased significantly for the whole cohort from baseline to year one and remained elevated at year 2 (Table 2). The Chemo group had a large mean increase in tingling/numbness at year 1 and 2 (30-32 units), significantly larger than the increase in the Hyst group (5-6 units, $P < 0.001$ between groups at year 1 and 2) (Figure 2, Supplementary table 4). Significant between-group differences were also found for lymphedema at year 1, with a moderate increase of 11 units in the Chemo group

compared to 2 (trivial) in the Hyst group ($P = 0.007$). There were no between-group differences in symptom scales between the Hyst group and the LNS group.

Development of treatment-related symptoms

Overall, 76% of patients reported no moderate/severe lymphedema symptoms at any timepoint (Figure 3A). Preoperatively, 10% of patients reported moderate/severe lymphedema symptoms, while an additional 13% reported moderate/severe symptoms that debuted post-operatively. Of 27 patients reporting moderate/severe lymphedema symptoms at year 1, 12 had reported moderate/severe symptoms at baseline (Figure 3B). Debut of moderate/severe lymphedema symptoms at year 1 were reduced/resolved in a third of patients at year 2. At year two, 12/28 of patients reporting lymphedema had previously reported no/light symptoms.

At baseline 7% of all patients reported moderate/severe tingling/numbness, while 19% of patients reported debut at year 1 and/or year 2 (Figure 3C). At year 1, 27 of 30 patients reporting moderate/severe tingling/numbness symptoms had reported no/light symptoms at baseline (Figure 3D). Of these 27, 16 reported persisting moderate/severe symptoms at year 2, the majority (14) being from the Chemo group.

Treatment-specific effect on patient-reported outcome

In linear mixed regression models (Figure 4, Full data in Supplementary table 5) adjuvant chemotherapy had an independent negative effect on physical function (regression coefficient -7.5, 95% CI -11.6 to -3.4, $P < 0.001$) and social function (-9.3, 95% CI -14.7 to -3.8, $P = 0.002$; Figure 4A).

For symptom scales (Figure 4B), adjuvant chemotherapy had a large increasing (detrimental) effect on tingling/numbness (regression coefficient 27.1, 95% CI 20.1-34.2, $P < 0.001$) and smaller increasing effects on fatigue (6.9, 95% CI 0.9-12.9, $P = 0.025$), lymphedema (8.9, 95% CI 3.6-14.2, $P = 0.001$) and taste change (5.0, 95% CI 0.7-9.3, $P = 0.024$). No effects of LNS or surgical modality were identified in the models. There were no relevant time-treatment interactions between year 1 and 2 post-treatment, thus effects of treatment were considered stable over this period (Supplementary table 5).

As it may be argued that patients undergoing sentinel node biopsy have a risk of morbidity more similar to non-lymphadenectomized patients than to those undergoing lymphadenectomy, this was explored in separate models. Grouping unstaged patients with those who had undergone sentinel node biopsy and comparing these with patients undergoing

lymphadenectomy, did not identify any significant effect on lymphedema score or alter estimates for adjuvant chemotherapy (Supplementary table 6).

COMMENT

Principal findings

We present, to our knowledge, the largest study prospectively investigating PROs in patients treated with no LNS for low-risk disease and adjuvant chemotherapy for high-risk disease, largely omitting adjuvant radiotherapy. Overall, endometrial cancer patients had good post-treatment quality of life, functioned well and expressed few symptoms, but increases in tingling/numbness and lymphedema were identified at the cohort level. We found that patients undergoing adjuvant chemotherapy more often reported long-term neuropathy, lymphedema, and fatigue as well as inferior physical function. In contrast, among patients not undergoing chemotherapy, we found no differences between those undergoing LNS and those treated by hysterectomy and BSO alone.

Results in context of what is known

We demonstrate that endometrial cancer patients overall have good self-reported quality of life and functioning at one and two years post-treatment. At baseline, global health status/quality of life and emotional function were below the average population reference but increased with time in all treatment groups. These findings harmonize with previous prospective studies in endometrial cancer populations.[21-23] The observed mean increase of quality of life and functional scales could potentially be explained by low baseline scores due to a newly received cancer diagnosis with associated symptoms, anxiety and affection of quality-of-life domains.

Our study did not demonstrate a clear link between lymphedema and LNS. Increased lymphedema score was reported for the Chemo group, but not for the group treated with LNS without adjuvant chemotherapy. Although the proportion of sentinel node biopsy was higher in the LNS group, and the proportion of para-aortic lymphadenectomy was higher in the Chemo group, the total lymphadenectomy rates excluding sentinel node biopsy were similar for the two groups (73% vs 75%). Cross-sectional studies have reported significant mean increases in self-reported lymphedema scores in patients with lymphadenectomy compared to those without.[24, 25] Importantly, other conditions than lymph tissue removal can result in lymphedema, and likely have increasing impact at longer follow-up times, especially in an endometrial cancer population with high age and comorbidity burden. These factors, combined with specified time points for follow-up, correction for baseline values and avoidance of recall-bias could explain why results from longitudinal and cross-sectional studies may differ. Adjuvant chemotherapy is not an acknowledged risk factor for lymphedema in endometrial cancer patients.

Interestingly, in experimental models, paclitaxel inhibits neolymphangiogenesis, implying possible interference in the post-operative healing process.[26] In addition, adjuvant taxane-based chemotherapy has been implicated as a risk factor for arm lymphedema after breast cancer surgery with axillar node dissection, but clinical data is conflicting.[27, 28]

The increase in self-reported neuropathy after receiving adjuvant chemotherapy harmonizes with longitudinal studies on endometrial cancer patients receiving radiochemotherapy compared to either adjuvant modality alone.[22, 29] Our results further confirm this effect and provide novel data on the evolution of these symptoms over the first two postoperative years, with late debut of symptoms in some patients, and a substantial proportion of patients reporting unresolved symptoms at year two.

Clinical implications

We have identified treatment-specific changes in self-reported outcomes that are useful when counselling patients on adjuvant treatment, as this is a group with a high comorbidity load and varying life-expectancy. The main alternative approach for high-risk patients, adjuvant external beam radiotherapy, is not likely to cause neurological symptoms but instead causes long-term bowel symptoms, with remaining problems at follow-up after 10-15 years[7-9], thus the most promising approach to improving quality of life in endometrial cancer survivors is likely a further individualization of adjuvant treatment. We have recently reported that despite a substantial increase over time of adjuvant chemotherapy to early-stage/high-risk patients in a Norwegian tertiary hospital, survival and recurrence rates were unchanged for this group.[30] Further reduction of patients undergoing adjuvant chemotherapy may be achieved through better stratification, ideally by implementing new classifiers such as imaging biomarkers or molecular subgroups (e.g. TCGA/ProMisE) in treatment planning for these patients[31, 32], as well as developing and making available novel therapeutic agents to replace traditional chemotherapy where possible.

Research implications

Self-assessed lymphedema did not associate to LNS in our study. Whether this is attributable to measurement tool issues, prompt and effective treatment of lymphedema, patient adaptation, or cultural differences in reporting symptoms would be interesting to explore in future studies. Due to insufficient data, we were unable to explore the effect of SNL subgroups on PROs, and data on this is still mainly lacking.[33] Finalizing inclusion and maturation of MoMaTEC2 data

will provide better insight into the effect of different LNS techniques and long-term evolution of associated symptoms.

Strengths and limitations

Our study has several strengths. The importance of prospective registration for PROs should be stressed, as the baseline values areimportant determinators for long-term PROs. Previous studies have identified age, body mass index, comorbidity, tumour stage and marital and socioeconomic status to be important predictors of PROs in endometrial cancer[21, 23, 34], and these can be approximated by including baseline PRO values. We also limited our analyses to non-relapsing survivors thereby excluding bias introduced by successive treatments and changes in prognosis. PROs for patients with progressive and recurrent disease is likely to differ from the results of our study, and research questions and assessment approaches should be different for these groups.

Our results may be biased by the fact that treatment is not randomized but based on risk-assessment, leading to unbalanced clustering of treatment modalities such as more comprehensive lymphadenectomy performed in patients receiving chemotherapy. We have attempted to handle this through mixed model analysis, but few included patients receiving chemotherapy without LNS may to some degree influence the isolated PRO effects when comparing chemotherapy and lymph surgery.

Conclusions

We find that endometrial cancer patients undergoing LNS without receiving chemotherapy are comparable to those not undergoing LNS and do not experience any significant deterioration from baseline to year 1 and 2, whereas patients receiving adjuvant chemotherapy have a higher risk of experiencing long-term neuropathy, lymphedema, and fatigue as well as inferior physical function. Considering these data, further striving to individualize adjuvant treatment is more pressing than adopting new surgical staging techniques.

ACKNOWLEDGEMENTS

We wish to express our gratitude to all participating patients, to the clinical team at Department of Obstetrics and Gynecology, HUS and especially to Elisabeth Enge, Olivera Bozickovic and Kadri Madissoo for assistance in inclusion and follow-up of patients. These persons receive salary from Helse Vest and the University of Bergen for their work in the research group.

ROLE OF THE FUNDING SOURCE

This work was supported by Helse Vest (F-12542), the University of Bergen, the Norwegian Cancer Society (190202-2017) and the Research Council of Norway (273280 and 239840).

CONFLICT OF INTEREST STATEMENT

Authors state no conflict of interest.

REFERENCES

1. FERLAY J, ERVIK M, LAM F, et al. Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer, 2020.
2. LU KH, BROADDUS RR. Endometrial Cancer. N Engl J Med 2020;383:2053-64.
3. JANDA M, GEBSKI V, BRAND A, et al. Quality of life after total laparoscopic hysterectomy versus total abdominal hysterectomy for stage I endometrial cancer (LACE): a randomised trial. The Lancet Oncology 2010;11:772-80.
4. WALKER JL, PIEDMONTE MR, SPIRTOS NM, et al. Laparoscopy Compared With Laparotomy for Comprehensive Surgical Staging of Uterine Cancer: Gynecologic Oncology Group Study LAP2. Journal of Clinical Oncology 2009;27:5331-36.
5. HELGERS RJA, WINKENS B, SLANGEN BFM, WERNER HMJ. Lymphedema and Post-Operative Complications after Sentinel Lymph Node Biopsy versus Lymphadenectomy in Endometrial Carcinomas-A Systematic Review and Meta-Analysis. J Clin Med 2020;10.
6. LEITAO MM, JR., ZHOU QC, GOMEZ-HIDALGO NR, et al. Patient-reported outcomes after surgery for endometrial carcinoma: Prevalence of lower-extremity lymphedema after sentinel lymph node mapping versus lymphadenectomy. Gynecologic oncology 2020;156:147-53.
7. DE BOER SM, NOUT RA, JÜRGENLIEMK-SCHULZ IM, et al. Long-Term Impact of Endometrial Cancer Diagnosis and Treatment on Health-Related Quality of Life and Cancer Survivorship: Results From the Randomized PORTEC-2 Trial. Int J Radiat Oncol Biol Phys 2015;93:797-809.
8. NOUT RA, PUTTER H, JÜRGENLIEMK-SCHULZ IM, et al. Five-year quality of life of endometrial cancer patients treated in the randomised Post Operative Radiation Therapy in Endometrial Cancer (PORTEC-2) trial and comparison with norm data. European journal of cancer (Oxford, England : 1990) 2012;48:1638-48.

9. NOUT RA, VAN DE POLL-FRANSE LV, LYBEERT ML, et al. Long-term outcome and quality of life of patients with endometrial carcinoma treated with or without pelvic radiotherapy in the post operative radiation therapy in endometrial carcinoma 1 (PORTEC-1) trial. J Clin Oncol 2011;29:1692-700.
10. RANDALL ME, FILIACI VL, MUSS H, et al. Randomized Phase III Trial of Whole-Abdominal Irradiation Versus Doxorubicin and Cisplatin Chemotherapy in Advanced Endometrial Carcinoma: A Gynecologic Oncology Group Study. Journal of Clinical Oncology 2006;24:36-44.
11. MAGGI R, LISSONI A, SPINA F, et al. Adjuvant chemotherapy vs radiotherapy in high-risk endometrial carcinoma: results of a randomised trial. Br J Cancer 2006;95:266-71.
12. SUSUMU N, SAGAE S, UDAGAWA Y, et al. Randomized phase III trial of pelvic radiotherapy versus cisplatin-based combined chemotherapy in patients with intermediate- and high-risk endometrial cancer: a Japanese Gynecologic Oncology Group study. Gynecologic oncology 2008;108:226-33.
13. STUKAN M, ZALEWSKI K, MARDAS M, et al. Independent psychometric validation of European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Endometrial Cancer Module (EORTC QLQ-EN24). Eur J Cancer Care (Engl) 2018;27.
14. GREIMEL E, NORDIN A, LANCELEY A, et al. Psychometric validation of the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Endometrial Cancer Module (EORTC QLQ-EN24). European journal of cancer (Oxford, England : 1990) 2011;47:183-90.
15. FOSSA SD, HESS SL, DAHL AA, HJERMSTAD MJ, VEENSTRA M. Stability of health-related quality of life in the Norwegian general population and impact of chronic morbidity in individuals with and without a cancer diagnosis. Acta oncologica (Stockholm, Sweden) 2007;46:452-61.
16. FAYERS PM AN, BJORDAL K, GROENVOLD M, CURRAN D, BOTTOMLEY A. The EORTC QLQ-C30 Scoring Manual (3rd Edition). Brussels: European Organisation for Research and Treatment of Cancer, 2001.
17. COHEN J. *Statistical power analysis for the behavioral sciences.* New York: Lawrence Erlbaum Associates; 1988;8-27.
18. NORMAN GR, SLOAN JA, WYRWICH KW. Interpretation of Changes in Health-related Quality of Life: The Remarkable Universality of Half a Standard Deviation. Medical Care 2003;41.

19. COCKS K, KING MT, VELIKOVA G, et al. Evidence-based guidelines for interpreting change scores for the European Organisation for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30. European journal of cancer (Oxford, England : 1990) 2012;48:1713-21.
20. COENS C, PE M, DUECK AC, et al. International standards for the analysis of quality-of-life and patient-reported outcome endpoints in cancer randomised controlled trials: recommendations of the SISAQOL Consortium. The Lancet Oncology 2020;21:e83-e96.
21. FERRANDINA G, PETRILLO M, MANTEGNA G, et al. Evaluation of quality of life and emotional distress in endometrial cancer patients: A 2-year prospective, longitudinal study. Gynecologic oncology 2014;133:518-25.
22. DE BOER SM, POWELL ME, MILESHKIN L, et al. Toxicity and quality of life after adjuvant chemoradiotherapy versus radiotherapy alone for women with high-risk endometrial cancer (PORTEC-3): an open-label, multicentre, randomised, phase 3 trial. The Lancet Oncology 2016;17:1114-26.
23. ZANDBERGEN N, DE ROOIJ BH, VOS MC, et al. Changes in health-related quality of life among gynecologic cancer survivors during the two years after initial treatment: a longitudinal analysis. Acta oncologica (Stockholm, Sweden) 2019;58:790-800.
24. ANGIOLI R, PLOTTI F, CAFA EV, et al. Quality of life in patients with endometrial cancer treated with or without systematic lymphadenectomy. European journal of obstetrics, gynecology, and reproductive biology 2013;170:539-43.
25. VAN DE POLL-FRANSE LV, PIJNENBORG JM, BOLL D, et al. Health related quality of life and symptoms after pelvic lymphadenectomy or radiotherapy vs. no adjuvant regional treatment in early-stage endometrial carcinoma: a large population-based study. Gynecologic oncology 2012;127:153-60.
26. ZAMORA A, ALVES M, CHOLLET C, et al. Paclitaxel induces lymphatic endothelial cells autophagy to promote metastasis. Cell Death & Disease 2019;10:956.
27. CARIATI M, BAINS SK, GROOTENDORST MR, et al. Adjuvant taxanes and the development of breast cancer-related arm lymphoedema. British Journal of Surgery 2015;102:1071-78.
28. SWAROOP MN, FERGUSON CM, HORICK NK, et al. Impact of adjuvant taxane-based chemotherapy on development of breast cancer-related lymphedema: results from a large prospective cohort. Breast Cancer Res Treat 2015;151:393-403.

29. MATEI D, FILIACI V, RANDALL ME, et al. Adjuvant Chemotherapy plus Radiation for Locally Advanced Endometrial Cancer. N Engl J Med 2019;380:2317-26.
30. FORSSE D, BERG HF, BOZICKOVIC O, et al. Maintained survival outcome after reducing lymphadenectomy rates and optimizing adjuvant treatment in endometrial cancer. Gynecologic oncology 2020.
31. KOMMOSS S, MCCONECHY MK, KOMMOSS F, et al. Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. Annals of oncology : official journal of the European Society for Medical Oncology 2018;29:1180-88.
32. LEÓN-CASTILLO A, DE BOER SM, POWELL ME, et al. Molecular Classification of the PORTEC-3 Trial for High-Risk Endometrial Cancer: Impact on Prognosis and Benefit From Adjuvant Therapy. Journal of Clinical Oncology 2020;38:3388-97.
33. OBERMAIR HM, O'HARA M, OBERMAIR A, JANDA M. Paucity of data evaluating patient centred outcomes following sentinel lymph node dissection in endometrial cancer: A systematic review. Gynecol Oncol Rep 2021;36:100763.
34. SMITS A, LOPES A, BEKKERS R, GALAAL K. Body mass index and the quality of life of endometrial cancer survivors--a systematic review and meta-analysis. Gynecologic oncology 2015;137:180-7.

Table 1. Clinical and pathological characteristics of included patients

	Hyst group	LNS group	Chemo group	p (Kruskall-Wallis)
Included (n)	176	132	138	
Age at treatment (median/IQR)	67 (14)	66 (13)	69 (11)	0.129
Body mass index (median/IQR)	28.3 (8)	28.3 (7)	27.4 (7)	0.219
	n (%)	**n (%)**	**n (%)**	**p (Fischer exact test)**
Mode of surgery (hysterectomy)				<0.001
Laparotomy	16 (9)	40 (32)	88 (69)	
Robot-assisted laparoscopy	64 (37)	82 (66)	37 (29)	
Conventional laparoscopy	91 (53)	2 (2)	3 (2)	
Lymph node staging				<0.001
Not performed	177 (100)	0 (0)	20 (14)	
Sentinel node mapping	0 (0)	34 (26)	17 (12)	
Pelvic lymphadenectomy	0 (0)	86 (65)	47 (34)	
Para-aortic and pelvic	0 (0)	13 (10)	54 (39)	
Lymph node metastasis				<0.001
Not investigated	177 (100)	0 (0)	20 (14)	
Positive	0 (0)	0 (0)	30 (22)	
Negative	0 (0)	133 (100)	88 (64)	
FIGO stage				<0.001
I	172 (98)	133 (100)	72 (52)	
II	3 (2)	0 (0)	22 (16)	
III	1 (1)	0 (0)	40 (29)	
IV	0 (0)	0 (0)	4 (3)	
Histological group				<0.001
EEC Grade 1	110 (65)	72 (54)	12 (9)	
EEC Grade 2	50 (29)	52 (39)	26 (19)	
EEC Grade 3	5 (3)	5 (4)	32 (23)	
Non-endometrioid	5 (3)	4 (3)	68 (49)	
Recurrence within 2 years				0.039
Yes	5 (3)	6 (5)	13 (9)	
No	172 (97)	127 (95)	125 (91)	

Hyst group, Hysterectomy alone; LNS group, Hysterectomy with lymph node staging procedure; Chemo group: Hysterectomy with adjuvant chemotherapy, +/- LNS
IQR, Interquartile range; FIGO, International Federation of Gynecology and Obstetrics; EEC, Endometrioid endometrial cancer

Table 2. Overall cohort changes in EORTC scale means over time.

Functional scales[b]	Ref[a]	Baseline mean (sd)	Year 1 mean (sd)	ES	p	Year 2 mean (sd)	ES	p
Global health status/QoL	72	69 (22)	78 (20)	Small	**<0.001**	76 (23)	Small	**0.002**
Physical Function	80	87 (17)	86 (16)	Trivial	0.279	85 (19)	Trivial	0.115
Emotional Function	83	75 (21)	87 (18)	Moderate	**<0.001**	86 (18)	Moderate	**<0.001**
Cognitive Function	85	86 (19)	87 (18)	Trivial	0.686	86 (19)	Trivial	0.282
Social Function	85	82 (22)	89 (20)	Small	**<0.001**	88 (21)	Small	**0.011**
Sexual interest	-	13 (22)	19 (26)	Small	**<0.001**	20 (25)	Small	**<0.001**
Sexual activity	-	9 (19)	15 (24)	Small	**<0.001**	14 (23)	Small	**<0.001**
Sexual enjoyment	-	65 (22)	57 (28)	Small	0.514	55 (27)	Small	0.303
Symptomatic scales[c]								
Fatigue	29	26 (23)	24 (23)	Trivial	0.162	25 (26)	Trivial	0.862
Lymphoedema	-	10 (18)	15 (22)	Small	**<0.001**	14 (20)	Small	**0.003**
Urological symptoms	-	17 (19)	16 (18)	Trivial	0.715	15 (16)	Trivial	0.606
Gastrointestinal symptoms	-	16 (16)	14 (15)	Trivial	0.232	14 (15)	Trivial	0.503
Poor body image	-	9 (18)	8 (16)	Trivial	0.211	9 (19)	Trivial	0.655
Sexual/vaginal problems	-	16 (21)	20 (21)	Small	0.124	24 (24)	Small	0.054
Pain in back and pelvis	-	27 (29)	23 (28)	Trivial	**0.014**	23 (29)	Trivial	0.132
Tingeling/numbness	-	11 (22)	24 (30)	Moderate	**<0.001**	24 (29)	Moderate	**<0.001**
Muscular pain	-	26 (30)	30 (30)	Trivial	**0.026**	31 (30)	Trivial	**0.004**
Hair loss	-	9 (20)	6 (18)	Trivial	0.173	8 (19)	Trivial	0.338
Taste change	-	5 (14)	4 (15)	Trivial	0.611	6 (18)	Trivial	0.283

Wilcoxon signed rank analysis of difference in means between each follow-up time point and baseline. P-values <0.05 in bold.

EORTC, European Organisation for Research and Treatment of Cancer; QoL, Quality of life

ES: Effect size based on Cohen's d (Supl. Table 3)

a References are sex-specific, age-weighted means from an unselected Norwegian population (Fossa et al. 2007)

b Increasing means signify increased function

c Increasing means signify increased symptoms

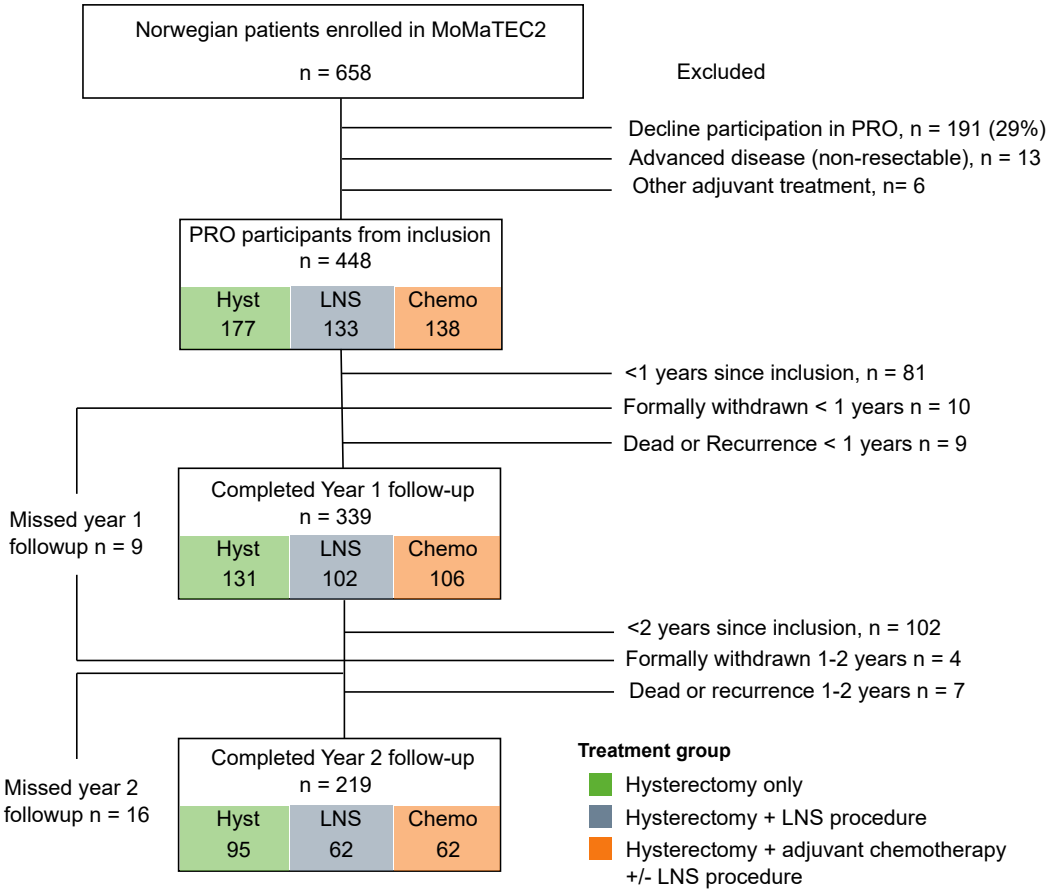

Figure 1. Patients assessed for eligibility and included in study at each follow-up time point. PRO – patient-reported outcomes, LNS - lymph node staging.

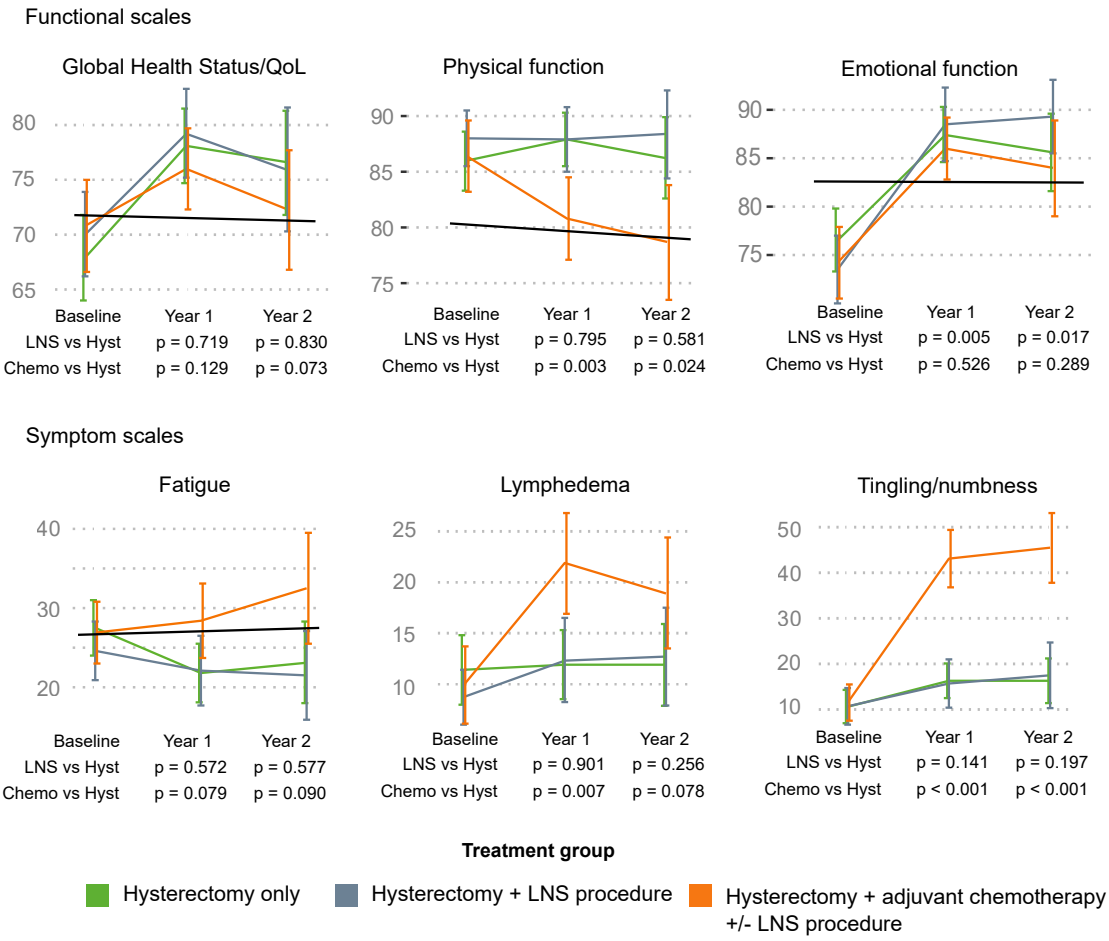

Figure 2. Patient reported mean EORTC scale scores with 95% confidence intervals. Increases in functional scales signify an increase in function, increases in symptom scales signify increase of symptom. Reference values (black lines) are age- and sex weighted means from a Norwegian general population survey (Available for EORTC QLQ-C30, Fossa et al. 2007). P-values are derived from Mann-Whitney test of change from baseline compared to Hyst group. Values of all analysed EORTC scales can be seen in supplementary table 4.

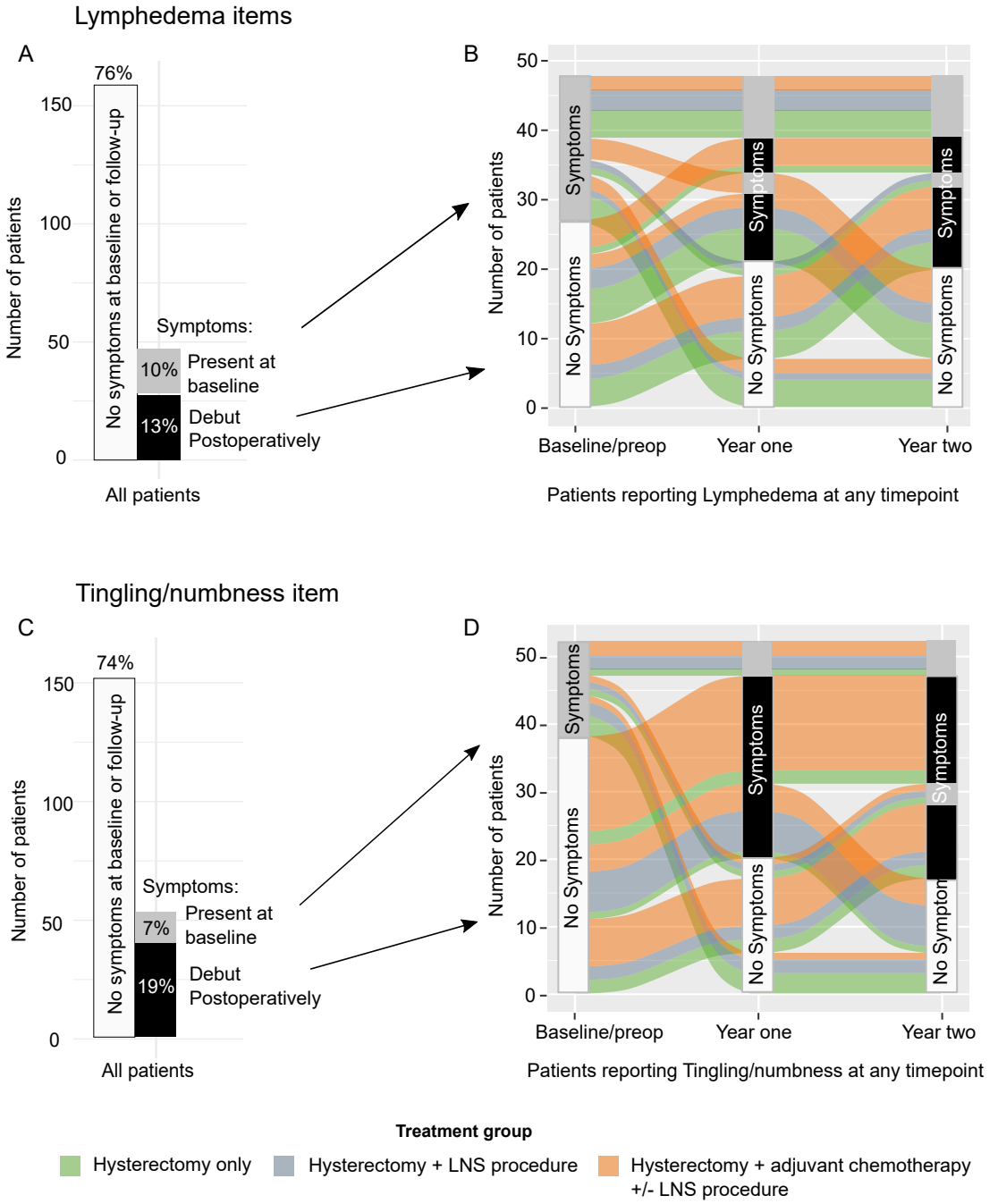

Figure 3. Case-wise analysis of treatment related symptoms in patients with complete 2 year follow-up data. A) Lymphedema symptoms defined as answering “quite a bit” or “very much” to either of the lymphedema associated items at any timepoint, in all patients (n=204). B) Case-wise evolution of lymphedema symptoms over time, by treatment received, only patients reporting symptoms are shown. C) Neuropathy symptoms defined as answering “quite a bit” or “very much” to the tingling/numbness item at any timepoint, in all patients (n=203). D) Case-wise evolution of tingling/numbness symptoms over time, only patients reporting symptoms are shown.

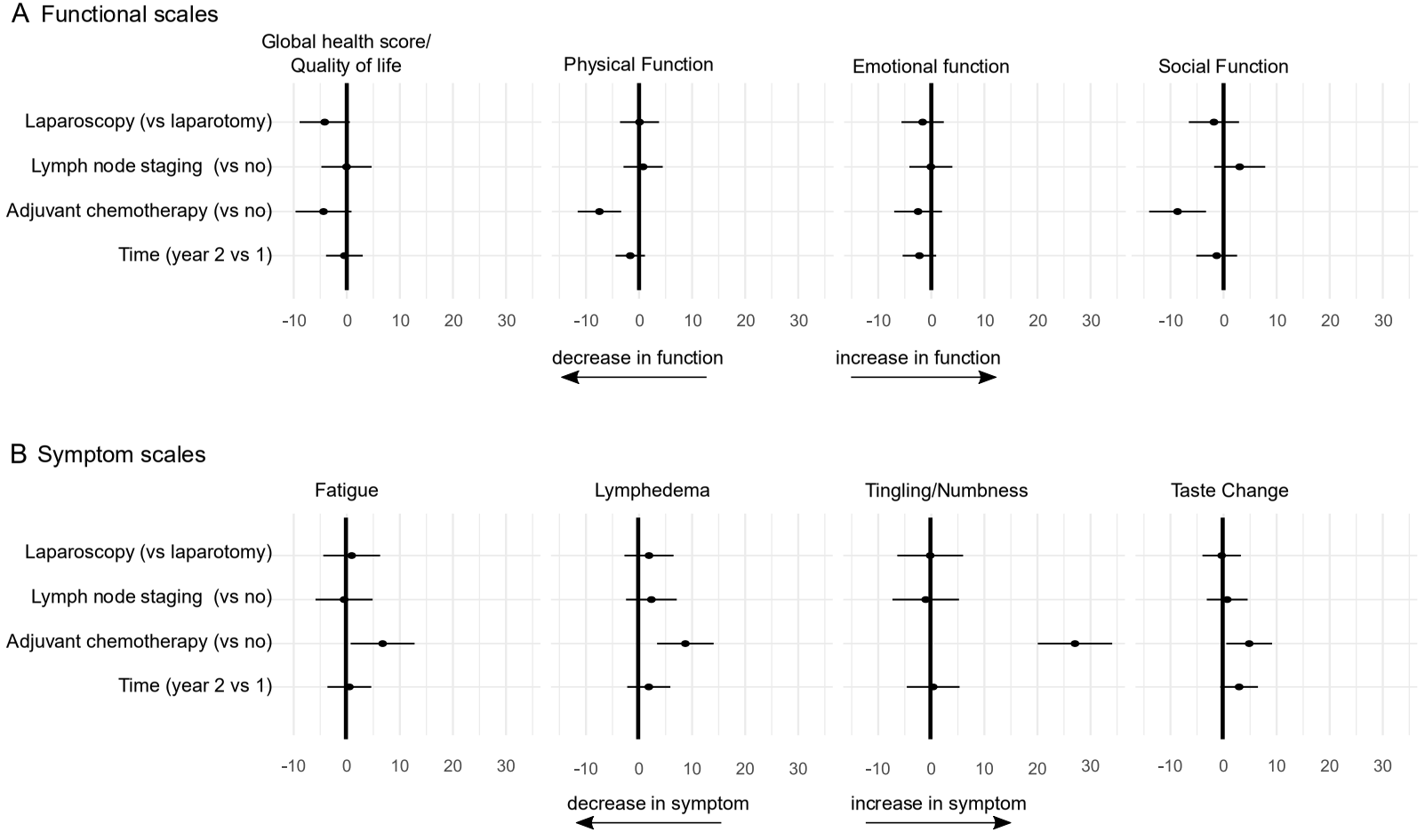

Figure 4. Effect estimates of time and treatment effects with 95% confidence intervals in linear mixed models for EORTC patient reported A) functional and B) symptom scales. Models are adjusted for baseline scores, all variables shown and interactions between time and chemotherapy and time and lymph node staging (LNS). Effect estimates for all analysed scales with p-values obtained can be seen in supplementary table 5.

Supplementary table 1. Clinical and pathological characteristics of the studied cohort compared to patients declining participation in patient reported outcome registration.

	Respondents	Non-respondents
Included (n)	467	191
Age at treatment (median/IQR)	68 (14)	68 (16)
Body mass index (median/IQR)	28 (8)	28 (7)
	n (%)	**n (%)**
Mode of surgery (hysterectomy)		
Laparotomy	152 (35)	77 (48)
Laparoscopy	185 (42)	41 (26)
Robot-assisted laparoscopy	101 (23)	43 (27)
Lymph node staging		
Not performed	203 (44)	102 (53)
Sentinel node mapping	52 (11)	5 (3)
Pelvic lymphadenectomy	140 (30)	56 (29)
Para-aortic and pelvic	70 (15)	28 (15)
Lymph node metastasis		
Not investigated	203 (44)	102 (53)
Positive	37 (8)	16 (8)
Negative	226 (49)	73 (38)
FIGO stage		
I	381 (82)	134 (75)
II	27 (6)	12 (7)
III	45 (10)	22 (12)
IV	12 (3)	11 (6)
Histology		
EEC Grade 1	197 (43)	72 (40)
EEC Grade 2	130 (28)	50 (28)
EEC Grade 3	46 (10)	20 (11)
Non-EEC	86 (19)	36 (20)
Adjuvant treatment		
None	313 (67)	113 (59)
External radiation	1 (0)	3 (2)
Brachytherapy	1 (0)	1 (1)
Chemotherapy	147 (32)	67 (35)
Hormonal treatment	3 (1)	3 (2)
Chemotherapy + radiation	1 (0)	2 (1)
Recurrence within 2 years		
Yes	25 (5)	16 (8)
No	429 (92)	151 (79)
Not completely resected at primary surgery	13 (3)	24 (13)

IQR: Interquartile range

FIGO: International Federation of Gynecology and Obstetrics

EEC: endometrioid endometrial cancer

Supplementary table 2. Number of responses per EORTC scale at each assessment time point

	Baseline	**Year 1**	**Year 2**
	n (%)	**n (%)**	**n (%)**
Eligible patients	448	367	237
Missing assessments	0 (0)	28 (8)	18 (8)
Respondents	448 (100%)	339 (92)	219 (92)
EORTC scales			
Global health status/ Quality of life	443 (99%)	338 (100%)	219 (100%)
Physical Function	447 (100%)	339 (100%)	219 (100%)
Emotional Function	443 (99%)	338 (100%)	219 (100%)
Cognitive Function	444 (99%)	338 (100%)	219 (100%)
Social Function	444 (99%)	338 (100%)	219 (100%)
Sexual interest	418 (93%)	333 (98%)	211 (96%)
Sexual activity	421 (94%)	333 (98%)	211 (96%)
Sexual enjoyment*	80 (18%)	109 (32%)	68 (31%)
Fatigue	446 (100%)	339 (100%)	219 (100%)
Lymphoedema	444 (99%)	336 (99%)	216 (99%)
Urological symptoms	444 (99%)	336 (99%)	216 (99%)
Gastrointestinal symptoms	443 (99%)	336 (99%)	216 (99%)
Poor body image	436 (97%)	334 (99%)	216 (99%)
Sexual/vaginal problems*	81 (18%)	110 (32%)	68 (31%)
Pain in back and pelvis	442 (99%)	335 (99%)	216 (99%)
Tingeling/numbness	443 (99%)	335 (99%)	216 (99%)
Muscular pain	441 (98%)	336 (99%)	215 (98%)
Hair loss	443 (99%)	335 (99%)	215 (98%)
Taste change	443 (99%)	336 (99%)	215 (98%)

EORTC: European Organisation for Research and Treatment of Cancer

* Only answered if the respondent has been sexually active during the last 4 weeks

Supplementary table 3. Cohen's d effect sizes for included EORTC scales as calculated based the study population baseline scores and compared to published anchor-based reference guidelines available for the EORTC-C30 questionnaire (Cocks et al. 2012)

	Questionnaire	SD	Study population baseline effect sizes			Anchor-based reference					
						Improvement			Deterioration		
Functional scales[1]			0.2 (small)	0.5 (moderate)	0.8 (large)	small	medium	large	small	medium	large
Global health status/ Quality of life	C30	22.4	4	11	18	5	8	-	5	10	16
Physical Function	C30	17.5	3	9	14	2	7	-	5	10	17
Emotional Function	C30	21.4	4	11	17	6	9	-	3	12	-
Cognitive Function	C30	18.7	4	9	15	3	7	-	1	7	-
Social Function	C30	21.7	4	11	17	3	8	-	6	11	-
Sexual Interest	EN24	21.9	4	11	17						
Sexual Activity	EN24	19.1	4	10	15						
Sexual Enjoyment	EN24	22.2	4	11	18						
Symptom scales[2]											
Fatigue	C30	22.8	5	11	18	4	9	-	5	10	15
Lymphoedema	EN24	18.3	4	9	15						
Urological symptoms	EN24	19.0	4	10	15						
Gastrointestinal symptoms	EN24	15.8	3	8	13						
Poor body image	EN24	18.5	4	9	15						
Sexual/vaginal problems	EN24	20.8	4	10	17						
Pain in back and pelvis	EN24	28.9	6	14	23						
Tingeling/numbness	EN24	22.0	4	11	18						
Muscular pain	EN24	30.0	6	15	24						
Hair loss	EN24	20.1	4	10	16						
Taste change	EN24	14.4	3	7	12						

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C30: general cancer questionnaire - 30 items

EN24: endometrial cancer questionnaire - 24 items

SD: standard deviation of score at baseline assessment

[1] Increasing means signify increased function

[2] Increasing means signify increased symptoms

Supplementary table 4. Changes in EORTC-scale means at year one and two compared to baseline mean score by treatment subgroup. Magnitude of changes assessed by effect size of the change (Cohen's d). Statistical comparison of change from baseline in treatment group compared to hysterectomy only group with Mann-Whitney test. p-values < 0.05 in bold.

	Hyst group				LNS group						Chemo group					
Scale	**Year 1**	**ES**	**Year 2**	**ES**	**Year 1**	**ES**	**p**	**Year 2**	**ES**	**p**	**Year 1**	**ES**	**p**	**Year 2**	**ES**	**p**
Functional scales[1]																
Global health status/ Quality of life	10	S	9	S	10	S	0.719	7	S	0.830	6	S	0.129	3	T	0.073
Physical Function	2	T	1	T	0	T	0.795	0	T	0.581	-6	S	**0.003**	-8	S	**0.024**
Emotional Function	11	M	9	S	15	M	**0.005**	16	M	**0.017**	12	M	0.526	10	S	0.289
Cognitive Function	3	T	2	T	2	T	0.749	2	T	0.907	0	T	0.961	-4	S	0.332
Social Function	9	S	7	S	10	S	0.868	8	S	0.471	1	T	0.131	-2	T	0.106
Sexual interest	7	S	6	S	6	S	0.751	9	S	0.278	6	S	0.919	5	S	0.382
Sexual activity	6	S	5	S	7	S	0.516	6	S	0.977	6	S	0.829	4	S	0.234
Sexual enjoyment	-7	S	-13	M	-11	M	0.559	-5	S	0.056	-9	S	0.832	-12	M	0.553
Symptom scales[2]																
Fatigue	-6	S	-4	T	-2	T	0.572	-3	T	0.577	1	T	0.079	6	S	0.090
Lymphoedema	2	T	2	T	3	T	0.901	4	S	0.256	11	M	**0.007**	8	S	0.078
Urological symptoms	-2	T	-4	S	0	T	0.865	-1	T	0.752	0	T	0.977	0	T	0.629
Gastrointestinal symptoms	-2	T	-2	T	-1	T	0.757	-2	T	0.281	-3	S	0.291	-2	T	0.782
Poor body image	-4	S	-2	T	-2	T	0.348	0	T	0.549	1	T	0.085	3	T	0.098
Sexual/vaginal problems	4	S	15	M	2	T	0.546	1	T	0.670	4	S	0.816	4	S	0.460
Pain in back and pelvis	-6	S	-7	S	-3	T	0.921	-1	T	0.712	-2	T	0.316	0	T	0.527
Tingeling/numbness	5	S	6	S	5	S	0.141	5	S	0.197	30	L	**<0.001**	32	L	**<0.001**
Muscular pain	4	T	5	T	2	T	0.835	6	S	0.765	4	T	0.589	2	T	0.351
Hair loss	-3	T	-2	T	-4	S	0.382	1	T	0.826	0	T	0.683	4	S	0.602
Taste change	-2	T	2	T	-2	T	0.662	0	T	0.318	1	T	0.496	6	S	0.554

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Hyst group: Hysterectomy alone, LNS group: hysterectomy with lymph node staging

Chemo group: hysterectomy with adjuvant chemotherapy, with or without LNS

ES - Effect size (Cohen's d): T: trivial S: small M: moderate L: large (Suppl Table 3)

[1] increasing means signify increased function

[2] increasing means signify increased symptoms

Supplementary table 5. Effect estimates of time and treatment effects in linear mixed models for EORTC scales. P-values obtained by Satterthwaites estimation of degrees of freedom.

	Baseline			Adjuvant Chemotherapy vs no			LNS vs no			Laparoscopy vs Laparotomy			Time (year 2 vs year 1)			Time:Chemo-therapy interaction			Time: LNS interaction		
Functional scale[1]	**EE**	**95% CI**	**p**	**EE**	**95% CI**	**p**	**EE**	**95% CI**	**p**	**EE**	**95% CI**	**p**	**EE**	**95% CI**	**p**	**EE**	**95% CI**	**p**	**EE**	**95% CI**	**p**
Global health status/ Quality of life	0.4	[0.3 : 0.5]	**<0.001**	-4.4	[-9.6 : 0.9]	0.104	-0.1	[-4.8 : 4.7]	0.982	-4.2	[-8.9 : 0.6]	0.086	-0.5	[-3.9 : 3]	0.798	-1.8	[-7.6 : 4]	0.545	-2.9	[-8.2 : 2.3]	0.279
Physical Function	0.5	[0.4 : 0.6]	**<0.001**	-7.5	[-11.6 : -3.4]	**<0.001**	0.8	[-2.9 : 4.5]	0.684	0.1	[-3.6 : 3.8]	0.961	-1.7	[-4.4 : 1.1]	0.243	1.1	[-3.5 : 5.7]	0.640	-0.3	[-4.5 : 4]	0.906
Emotional Function	0.3	[0.3 : 0.4]	**<0.001**	-2.5	[-7.0 : 2.0]	0.279	-0.1	[-4.1 : 4]	0.970	-1.6	[-5.6 : 2.4]	0.423	-2.2	[-5.4 : 0.9]	0.165	-1.6	[-6.9 : 3.7]	0.554	2.3	[-2.5 : 7.2]	0.340
Cognitive Function	0.5	[0.4 : 0.6]	**<0.001**	-3.5	[-8.1 : 1.2]	0.143	-1.6	[-5.7 : 2.6]	0.458	-1.6	[-5.6 : 2.5]	0.448	-1.7	[-5.1 : 1.7]	0.329	-1.1	[-6.8 : 4.5]	0.691	1.4	[-3.7 : 6.5]	0.592
Social Function	0.3	[0.2 : 0.4]	**<0.001**	-8.7	[-14 : -3.3]	**0.002**	3.0	[-1.8 : 7.9]	0.217	-1.8	[-6.5 : 2.9]	0.453	-1.3	[-5.1 : 2.6]	0.511	2.6	[-3.9 : 9]	0.433	-2.7	[-8.6 : 3.2]	0.367
Sexual Interest	0.6	[0.5 : 0.7]	**<0.001**	-1.1	[-7.6 : 5.4]	0.739	1.2	[-4.8 : 7.1]	0.699	-1.4	[-7.3 : 4.5]	0.644	0.2	[-3.8 : 4.2]	0.914	-2.7	[-9.2 : 3.9]	0.428	0.5	[-5.5 : 6.5]	0.876
Sexual Activity	0.6	[0.5 : 0.7]	**<0.001**	-0.8	[-7.0 : 5.3]	0.787	2.5	[-3 : 8]	0.376	1.2	[-4.5 : 6.8]	0.688	0.1	[-3.4 : 3.6]	0.961	0.2	[-5.5 : 6]	0.939	-3.1	[-8.3 : 2.1]	0.248
Sexual Enjoyment	0.6	[0.5 : 0.7]	**<0.001**	-1.1	[-7.6 : 5.4]	0.739	1.2	[-4.8 : 7.1]	0.699	-1.4	[-7.3 : 4.5]	0.644	0.2	[-3.8 : 4.2]	0.914	-2.7	[-9.2 : 3.9]	0.428	0.5	[-5.5 : 6.5]	0.876
Symptom scale[2]																					
Fatigue	0.4	[0.3 : 0.5]	**<0.001**	6.9	[0.9 : 12.9]	**0.025**	-0.3	[-5.7 : 5]	0.899	1.1	[-4.3 : 6.4]	0.690	0.6	[-3.5 : 4.8]	0.760	1.0	[-5.9 : 7.9]	0.776	-0.7	[-7 : 5.6]	0.831
Lymphoedema	0.5	[0.4 : 0.7]	**<0.001**	8.9	[3.6 : 14.2]	**0.001**	2.5	[-2.3 : 7.3]	0.308	2.0	[-2.6 : 6.7]	0.388	2.0	[-2.1 : 6.1]	0.336	-2.1	[-8.9 : 4.7]	0.552	-3.9	[-10.1 : 2.2]	0.213
Urological symptoms	0.4	[0.4 : 0.5]	**<0.001**	0.3	[-4.1 : 4.6]	0.908	-0.6	[-4.4 : 3.3]	0.775	0.2	[-3.7 : 4]	0.932	-2.1	[-5 : 0.9]	0.166	0.4	[-4.5 : 5.3]	0.874	2.1	[-2.4 : 6.5]	0.361
Gastrointestinal symptoms	0.6	[0.5 : 0.7]	**<0.001**	0.8	[-2.8 : 4.5]	0.651	-0.4	[-3.6 : 2.9]	0.820	2.5	[-0.7 : 5.7]	0.127	0.1	[-2.5 : 2.7]	0.929	1.3	[-3 : 5.6]	0.561	0.8	[-3.1 : 4.7]	0.694
Poor body image	0.3	[0.2 : 0.4]	**<0.001**	3.4	[-1.3 : 8.1]	0.153	0.7	[-3.5 : 4.9]	0.736	2.0	[-2.3 : 6.3]	0.365	1.7	[-0.7 : 4.2]	0.169	0.3	[-3.8 : 4.5]	0.876	-0.4	[-4.1 : 3.4]	0.848
Sexual/vaginal problems	0.5	[0.4 : 0.6]	**0.009**	4.8	[-1.1 : 10.7]	0.108	-4.6	[-9.9 : 0.7]	0.088	-0.2	[-5.4 : 5]	0.943	1.0	[-3.2 : 5.1]	0.652	-1.2	[-8.1 : 5.7]	0.731	3.1	[-3.2 : 9.4]	0.332
Pain in back and pelvis	0.4	[0.3 : 0.5]	**<0.001**	2.1	[-5 : 9.2]	0.565	1.2	[-5.2 : 7.7]	0.706	-1.7	[-7.8 : 4.3]	0.572	-0.2	[-6.1 : 5.6]	0.943	-1.1	[-10.8 : 8.7]	0.832	2.4	[-6.4 : 11.3]	0.587
Tingeling/numbness	0.4	[0.3 : 0.5]	**<0.001**	27.2	[20.2 : 34.2]	**<0.001**	-0.9	[-7.2 : 5.4]	0.782	0.0	[-6.2 : 6.2]	0.989	0.5	[-4.5 : 5.5]	0.838	1.6	[-6.7 : 9.9]	0.711	-1.4	[-8.9 : 6.2]	0.722
Muscular pain	0.4	[0.3 : 0.5]	**<0.001**	2.7	[-4.8 : 10.3]	0.477	-1.9	[-8.7 : 4.9]	0.579	1.2	[-5.1 : 7.6]	0.705	0.2	[-6.1 : 6.5]	0.960	-5.4	[-15.8 : 5]	0.313	4.9	[-4.6 : 14.4]	0.313
Hair loss	0.3	[0.2 : 0.4]	**<0.001**	3.7	[-1.3 : 8.7]	0.148	-3.3	[-7.7 : 1.2]	0.148	1.5	[-2.8 : 5.8]	0.484	0.1	[-3.9 : 4]	0.975	-2.9	[-9.5 : 3.6]	0.384	3.9	[-2 : 9.9]	0.198
Taste change	0.1	[0 : 0.2]	0.151	5.0	[0.7 : 9.3]	**0.024**	0.8	[-3 : 4.7]	0.665	-0.2	[-3.8 : 3.4]	0.916	3.1	[-0.5 : 6.6]	0.089	0.8	[-5.1 : 6.7]	0.796	-2.2	[-7.6 : 3.2]	0.421

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EE effect estimate, CI Confidence interval, LNS lymph node staging (including sentinel node biopsy)

[1] positive effect estimate signifies increased function

[2] positive effect estimate signifies increased symptoms

Supplementary table 6. Linear mixed model effect estimates of time and treatment effects for EORTC scales, with alternate grouping of lymph node staging procedures. Here, SLN was grouped with no lymph node staging and compared to lymphadenectomy (pelvic +/- paraaortic). P-values obtained by Satterthwaites estimation of degrees of freedom.

	Baseline			Adjuvant Chemotherapy			LA versus SLN or no staging			Laparoscopy vs Laparotomy			Time (year 2 vs year 1)			Time: Chemo-therapy interaction			Time: LNS interaction		
Functional scale	**EE**	**95% CI**	**p**	**EE**	**95% CI**	**p**	**EE**	**95% CI**	**p**	**EE**	**95% CI**	**p**	**EE**	**95% CI**	**p**	**EE**	**95% CI**	**p**	**EE**	**95% CI**	**p**
Global health status/ Quality of life	0.4	[0.3 : 0.5]	**<0.001**	-4.5	[-9.7 : 0.6]	0.083	-0.4	[-5.5 : 4.6]	0.876	-4.8	[-10 : 0.4]	0.069	-0.3	[-3.5 : 2.9]	0.869	-1.2	[-6.9 : 4.4]	0.673	-4.4	[-9.6 : 0.8]	0.099
Physical Function	0.5	[0.4 : 0.6]	**<0.001**	-7.2	[-11.2 : -3.2]	**<0.001**	-0.7	[-4.7 : 3.3]	0.737	-0.7	[-4.7 : 3.4]	0.751	-1.4	[-4 : 1.2]	0.284	1.6	[-3 : 6.1]	0.494	-1.3	[-5.5 : 2.9]	0.543
Emotional Function	0.3	[0.3 : 0.4]	**<0.001**	-2.1	[-6.5 : 2.2]	0.337	-2.1	[-6.5 : 2.2]	0.334	-2.7	[-7 : 1.7]	0.231	-1.8	[-4.7 : 1.1]	0.234	-1.0	[-6.2 : 4.2]	0.698	1.4	[-3.4 : 6.2]	0.574
Cognitive Function	0.5	[0.4 : 0.6]	**<0.001**	-3.4	[-7.9 : 1.1]	0.136	-3.2	[-7.6 : 1.2]	0.160	-2.7	[-7.1 : 1.7]	0.226	-1.4	[-4.6 : 1.7]	0.368	-0.9	[-6.4 : 4.7]	0.754	1.0	[-4.1 : 6.1]	0.707
Social Function	0.3	[0.2 : 0.4]	**<0.001**	-7.8	[-13.1 : -2.6]	**0.030**	-0.1	[-5.3 : 5]	0.966	-3.0	[-8.1 : 2.2]	0.266	-1.4	[-5 : 2.1]	0.428	2.8	[-3.5 : 9.2]	0.381	-3.3	[-9.1 : 2.5]	0.267
Sexual Interest	0.6	[0.5 : 0.7]	**<0.001**	-0.6	[-7 : 5.7]	0.846	-0.3	[-6.7 : 6.1]	0.931	-1.8	[-8.3 : 4.7]	0.595	0.1	[-3.6 : 3.8]	0.950	-2.8	[-9.3 : 3.7]	0.399	0.9	[-5.1 : 6.8]	0.778
Sexual Activity	0.6	[0.5 : 0.7]	**<0.001**	-0.2	[-6.2 : 5.9]	0.961	0.7	[-5.3 : 6.6]	0.829	1.0	[-5.2 : 7.1]	0.762	-0.8	[-4.1 : 2.4]	0.614	-0.8	[-6.5 : 5]	0.793	-1.0	[-6.3 : 4.3]	0.708
Sexual Enjoyment	0.6	[0.3 : 0.9]	**<0.001**	0.1	[-15.9 : 16.1]	0.992	-7.7	[-26.4 : 11.1]	0.428	-19.9	[-39.2 : -0.5]	0.050	2.8	[-2 : 7.5]	0.267	-4.1	[-11.2 : 3.1]	0.276	-3.7	[-11 : 3.6]	0.334
Symptom scale																					
Fatigue	0.4	[0.3 : 0.5]	**<0.001**	7.1	[1.2 : 12.9]	**0.019**	-0.3	[-6.1 : 5.5]	0.914	1.4	[-4.5 : 7.3]	0.638	-0.2	[-4 : 3.6]	0.915	-0.3	[-7.1 : 6.5]	0.935	2.1	[-4.2 : 8.3]	0.518
Lymphoedema	0.5	[0.4 : 0.7]	**<0.001**	9.1	[3.9 : 14.3]	**<0.001**	2.7	[-2.4 : 7.9]	0.297	2.6	[-2.5 : 7.7]	0.317	1.4	[-2.3 : 5.2]	0.452	-2.5	[-9.2 : 4.3]	0.472	-3.4	[-9.5 : 2.8]	0.282
Urological symptoms	0.4	[0.4 : 0.5]	**<0.001**	0.1	[-4.1 : 4.3]	0.951	-0.7	[-4.9 : 3.4]	0.732	-0.2	[-4.4 : 4]	0.933	-1.4	[-4.1 : 1.3]	0.312	1.2	[-3.6 : 6.1]	0.619	0.4	[-4 : 4.9]	0.851
Gastrointestinal symptoms	0.6	[0.5 : 0.7]	**<0.001**	0.6	[-3 : 4.1]	0.748	1.1	[-2.4 : 4.6]	0.542	3.3	[-0.3 : 6.8]	0.072	0.1	[-2.2 : 2.5]	0.910	1.2	[-3.1 : 5.5]	0.579	1.0	[-2.9 : 4.9]	0.612
Poor body image	0.3	[0.2 : 0.4]	**<0.001**	2.3	[-2.2 : 6.9]	0.320	5.8	[1.3 : 10.3]	**0.012**	4.1	[-0.6 : 8.8]	0.089	2.8	[0.6 : 5.1]	**0.014**	2.1	[-1.9 : 6.1]	0.312	-4.2	[-7.9 : -0.5]	**0.026**
Sexual/vaginal problems	0.4	[0.1 : 0.7]	**0.012**	-6.0	[-20.7 : 8.7]	0.427	8.2	[-8.8 : 25.1]	0.350	-4.4	[-22 : 13.2]	0.629	3.2	[-4.1 : 10.4]	0.401	0.7	[-10.9 : 12.3]	0.904	-3.2	[-15.4 : 9]	0.612
Pain in back and pelvis	0.4	[0.3 : 0.5]	**<0.001**	2.7	[-4.2 : 9.7]	0.440	-0.5	[-7.3 : 6.4]	0.894	-1.9	[-8.5 : 4.7]	0.571	-0.4	[-5.7 : 5]	0.897	-1.4	[-10.9 : 8.2]	0.778	3.5	[-5.3 : 12.2]	0.436
Tingeling/numbness	0.4	[0.3 : 0.5]	**<0.001**	27.3	[20.4 : 34.1]	**<0.001**	-1.1	[-7.8 : 5.7]	0.759	-0.1	[-6.9 : 6.7]	0.971	-0.2	[-4.7 : 4.4]	0.942	0.6	[-7.6 : 8.8]	0.884	0.6	[-6.9 : 8.1]	0.874
Muscular pain	0.4	[0.3 : 0.5]	**<0.001**	2.5	[-4.9 : 9.8]	0.508	-0.3	[-7.5 : 7]	0.944	2.2	[-4.7 : 9.2]	0.531	0.2	[-5.6 : 6]	0.947	-5.9	[-16.2 : 4.3]	0.258	6.5	[-2.9 : 15.9]	0.180
Hair loss	0.3	[0.2 : 0.4]	**<0.001**	2.7	[-2.1 : 7.6]	0.272	-0.2	[-5 : 4.6]	0.933	2.4	[-2.3 : 7.1]	0.314	0.8	[-2.8 : 4.4]	0.667	-2.4	[-8.9 : 4.1]	0.472	2.9	[-3 : 8.9]	0.332
Taste change	0.1	[0 : 0.2]	0.140	5.1	[0.9 : 9.3]	**0.017**	1.3	[-2.8 : 5.4]	0.525	0.5	[-3.5 : 4.5]	0.801	2.2	[-1.1 : 5.4]	0.192	-0.4	[-6.2 : 5.4]	0.896	0.2	[-5.1 : 5.5]	0.941

EORTC: European Organisation for Research and Treatment of Cancer

EE effect estimate, CI Confidence interval, LA lymphadenectomy, SLN Sentinel lymph node biopsy, GI Gastrointestinal

[1] positive effect estimate signifies increased function

[2] positive effect estimate signifies increased symptoms

Appendix A

Treatment in MoMaTEC2

Standard treatment was hysterectomy with bilateral salpingo-oophorectomy (BSO). In algorithm-adhering centers, lymphadenectomy was omitted in patients with low-risk disease (endometrioid histology grade 1-2 in preoperative biopsy and grade 3 if less than 50% myometrial invasion on imaging) with immunohistochemical estrogen and progesterone receptor (ER/PR) positive expression in the preoperative endometrial sample. In the case of ER/PR negativity in otherwise low-risk patients a pelvic lymphadenectomy was performed. The level of immunohistochemical expression was revised in 2019 following an interim analysis comparing research-derived expression levels to routinely reported levels. The original cutoff <1% for ER and <10% for PR was changed to <30% for both, after consulting the MoMaTEC2 advisory board and participating centers.

Pelvic and para-aortic lymphadenectomy was routinely performed in high-risk patients: Endometrioid grade 3 with deep myometrial infiltration, any non-endometrioid histology or suspicion of FIGO stage >I (Imaging, preoperative clinical status, perioperative findings). Omentectomy was performed in patients with serous and clear cell histology. In control centers, sentinel node biopsy was performed for all risk groups, with hemipelvic lymphadenectomy in case of failed mapping. Mode of surgery (laparotomy, laparoscopy or robot-assisted laparoscopy) varied within and between centers.

Adjuvant treatment

MoMaTEC2 does not require a certain adjuvant therapy policy to be followed. Adjuvant treatment policy is however conform in Norway and advocates use of chemotherapy rather than radiotherapy. According to national guidelines, no adjuvant treatment is given to patients with endometrioid histology tumors and final FIGO I except IB with grade 3 differentiation. For

patients deemed at high risk postoperatively (FIGO IB endometrioid grade 3, any non-endometrioid histology, or any FIGO stage > I), standard treatment is 6 rounds of carboplatin plus paclitaxel at 3-week intervals. The regimen could be shortened/altered due to patient status at the treating physician's discretion. For FIGO II with possible non-free resection margins brachytherapy can be considered.

Appendix A

Specimens of the European Organisation for research and treatment of cancer (EORTC) questionnaires used in this thesis. Reprinted with permission from the EORTC Quality of Life group.

For the validation paper for the EORTC-C30, see

Aaronson, N. K., et al. (1993). "The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology." J Natl Cancer Inst 85(5): 365-376.

For using questionnaires in research, please contact EORTC, Quality of Life department at

www.eortc.org/research_field/quality-of-life/

EORTC QLQ – EN24

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems.

During the past week:	Not at all	A little	Quite a bit	Very much
31. Have you had swelling in one or both legs?	1	2	3	4
32. Have you felt heaviness in one or both legs?	1	2	3	4
33. Have you had pain in your lower back and / or pelvis?	1	2	3	4
34. When you felt the urge to pass urine, did you have to hurry to get to the toilet?	1	2	3	4
35. Have you passed urine frequently?	1	2	3	4
36. Have you had leaking of urine?	1	2	3	4
37. Have you had pain or a burning feeling when passing urine?	1	2	3	4
38. When you felt the urge to move your bowels, did you have to hurry to get to the toilet?	1	2	3	4
39. Have you had any leakage of stools?	1	2	3	4
40. Have you been troubled by passing wind?	1	2	3	4
41. Have you had cramps in your abdomen?	1	2	3	4
42. Have you had a bloated feeling in your abdomen?	1	2	3	4
43. Have you had tingling or numbness in your hands or feet?	1	2	3	4
44. Have you had aches or pains in your muscles or joints?	1	2	3	4
45. Have you lost hair?	1	2	3	4
46. Has food and drink tasted differently from usual?	1	2	3	4

Please go on to the next page

During the past week:	Not at all	A little	Quite a bit	Very much
47. Have you felt physically less attractive as a result of your disease or treatment?	1	2	3	4
48. Have you felt less feminine as a result of your disease or treatment?	1	2	3	4

During the past 4 weeks:	Not at all	A little	Quite a bit	Very much
49. To what extent were you interested in sex?	1	2	3	4
50. To what extent were you sexually active?	1	2	3	4
Answer these questions only if you have been sexually active during the past 4 weeks:				
51. Has your vagina felt dry during sexual activity?	1	2	3	4
52. Has your vagina felt short and / or tight?	1	2	3	4
53. Have you had pain during sexual intercourse or other sexual activity?	1	2	3	4
54. Was sexual activity enjoyable for you?	1	2	3	4

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: |_|_|_|_|
Your birthdate (Day, Month, Year): |_|_|_|_|_|_|_|_|
Today's date (Day, Month, Year): 31 |_|_|_|_|_|_|_|_|

		Not at All	A Little	Quite a Bit	Very Much
1.	Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2.	Do you have any trouble taking a long walk?	1	2	3	4
3.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4.	Do you need to stay in bed or a chair during the day?	1	2	3	4
5.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:

		Not at All	A Little	Quite a Bit	Very Much
6.	Were you limited in doing either your work or other daily activities?	1	2	3	4
7.	Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8.	Were you short of breath?	1	2	3	4
9.	Have you had pain?	1	2	3	4
10.	Did you need to rest?	1	2	3	4
11.	Have you had trouble sleeping?	1	2	3	4
12.	Have you felt weak?	1	2	3	4
13.	Have you lacked appetite?	1	2	3	4
14.	Have you felt nauseated?	1	2	3	4
15.	Have you vomited?	1	2	3	4
16.	Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

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ISBN: 9788230846926 (print)
9788230842911 (PDF)