Den mätta dagen är aldrig störst. Den bästa dagen är en dag av törst.

Karin Boye

Long-term survival and prognostic factors in endometrial cancer

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ABSTRACT

Aims: The over-all aims of this thesis were to evaluate the associations between prognostic factors and excess mortality rate, between socioeconomic and immigrant status and incidence rate, in endometrioid (EEC) and non-endometrioid (NEC) endometrial carcinoma.

Material and methods: Study I-III were retrospective population-based cohort studies including women resident in a defined geographical area, with endometrial carcinoma. Data on clinicopathological variables were collected from the Western Swedish Healthcare Region Clinical Registry for Endometrial Cancer and the Swedish Quality Registry of Gynecologic Cancer. In study III, data on education and immigrant status were collected from the Swedish Registry of Education and the Statistics Sweden Population Registry.

Results: Cohort 2, had a decreased excess mortality rate compared to cohort 1, EMRR 0.62 (95% CI 0.44-0.87) in the NEC group. There was a significant difference in distribution of treatment in cohort 2 (p<0.001), with increased adjuvant chemotherapy in combination with radiotherapy. Excess mortality was not increased with presence of P53 overexpression, EMRR 1.53 (95% CI 0.79-2.97), s-phase fraction ≥8%, EMRR 1.31 (95% CI 0.68-2.53), and

aneuploidy, EMRR 1.79 (95% CI 0.89-3.24). In aneuploidy stage I, grade 2, 5-year relative survival was 0.88 (95% CI 0.78-0.96). Women, aged 50-74-years, with low level of education had higher incidence rate of stage II and III-IV EEC, IRR 1.65 (95% CI 1.13-2.42) and IRR 1.82 (1.33-2.49) compared to high level of education.

Conclusions: Clinical protocol used in cohort 2, NEC, was associated with decreased excess mortality. We did not find P53 overexpression, s-phase fraction $\geq 8\%$ or aneuploidy associated with increased excess mortality although aneuploidy identified women with impaired survival in stage I grade 2. Lower level of education was associated with increased incidence rates of stage II-IV EEC in 50-74-year-old women.

Keywords: excess mortality rate, EEC, NEC, incidence rate

SAMMANFATTNING PÅ SVENSKA

Livmoderkroppscancer är den 6:e vanligaste cancersjukdomen hos kvinnor i världen. I Sverige insjuknar i medeltal ca 1400 kvinnor per år. Överlevnaden i livmoderkroppscancer är god, efter 5 år lever ca 85% av de som drabbats jämfört med motsvarande befolkning, då en majoritet av de som drabbas upptäcks i ett tidigt skede. Behandling består av kirurg men även tilläggsbehandling i form av strålning och cellgifter. Prognostiska faktorer förutsäger en sjukdoms naturalförlopp. Dessa används för att dela in kvinnor med livmoderkroppscancer i olika riskgrupper, vilka styr vilken typ av behandling som är aktuell. Utbildningsnivå kan påverka individens kunskap om hälsosamma levnadsvanor och symptom på sjukdom. Rökning och fetma är vanligare i grupper med låg utbildningsnivå. Syftet med denna avhandling var att studera prognostiska faktorer och deras påverkan på överdödlighet i livmoderkroppscancer samt att studera om kvinnor med låg utbildningsnivå eller utländsk härkomst har fler antal nya fall av mer utbredd livmoderkroppscancer jämfört med kvinnor med hög utbildning eller svensk härkomst.

Kvinnor i Västra Götalandsregionen och norra Halland som diagnosticeras med livmoderkroppscancer registreras i ett kvalitetsregister vid Regionalt Cancercentrum Väst. Utifrån data registrerat i dessa kvalitetsregister beräknades och jämfördes överlevnaden i olika typer av livmoderkroppscancer mellan två olika vårdprogram som användes mellan 1995–2006 och 2006–2011 i studie I. 5-års överlevanden beräknades och jämfördes även för olika prognostiska faktorer; p53, s-fasfraktion eller DNA ploidi i studie II. I studie III, inhämtades även data från Statistiska centralbyrån, och antalet nya fall av livmoderkroppscancer med olika utbredning beräknades och jämfördes mellan grupper med olika utbildningsnivå och härkomst.

I studie I fann vi att gruppen med sämst prognos av livmoderkroppscancer och som behandlades med det vårdprogram som användes mellan 2006–2011 hade

en signifikant lägre överdödlighet jämfört med den grupp som behandlades mellan 1995–2006. Vi fann också att andelen av de olika behandlingarna skiljde sig mellan de olika tidsperioderna; för gruppen med sämst prognos av livmoderkroppscancer fick 16 procentenheter fler behandlingar med strålning och cellgifter efter kirurgi jämfört med tidigare. I studie II fann vi inte att kvinnor med överuttryck av p53, förhöjd s-fasfraktion eller aneuploid i tumören hade högre överdödlighet jämfört med kvinnor med tumörer utan p53 överuttryck, s-fasfraktion <8% eller diploidi. I en undergrupp av kvinnor med tumör begränsad till livmoderkroppen, och med hög till måttlig mognadsgrad av tumörcellerna, identifierade aneuploid en grupp med sämre 5-årsöverlevnad på 88%. I studie III fann vi att kvinnor i åldern 50–74 år med låg utbildningsnivå hade fler antal nya fall av livmoderkroppscancer som var utbredd bortom livmoderkroppen jämfört med kvinnor i samma åldersgrupp med hög utbildning.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I.   Svanvik, T. Population-based cohort study of the effect of endometrial cancer classification and treatment criteria on long-term survival. Int J Gynecol Obstet 2017; 138: 183-189. doi: 10.1002/ijgo.12214.

II.   Svanvik, T. A. DNA ploidy status, s-phase fraction, and p53 are not independent prognostic factors for survival in endometrioid endometrial carcinoma FIGO stage I-III. Int J Gynecol Cancer. 2019 Jan 2013 doi: 10.1136/ijgc-2018-000082. [Epub ahead of print]

III.   Svanvik, T. Sociodemographic disparities in stage-specific incidences of endometrial cancer: A registry-based study in west Sweden, 1995-2016. Accepted for publication in Acta Oncologica, 07-Feb- 2019.

CONTENT

ABBREVIATIONS

BMI   Body mass index

BSO   Bilateral salpingo-oophorectomy

CI   Confidence interval

DNA   Deoxyribonucleic acid

DSS   Disease-specific survival

EBRT   External beam radiotherapy

EC   Endometrial carcinoma

EEC   Endometrioid endometrial carcinoma

EMR   Excess mortality rate

EMRR   Excess mortality rate ratio

ER   Estrogen receptor

ESGO   European Society of Gynaecological Oncology

ESMO   European Society of Medical Oncology

ESTRO   European Society of Radiotherapy and Oncology

FIGO   International Federation of Gynecology and Obstetrics

FFPE   Formalin-fixed paraffin-embedded

G   Grade

G1   Gap phase 1

GDF-15   Plasma growth differentiation factor-15

GOG   Gynecologic Oncology Group

HE4   Human epididymis protein 4

HER-2/neu	Human epidermal growth factor receptor 2
HNPCC	Non-polyposis colon cancer
HR	Hazard ratio
IHC	Immunohistochemistry
INCA	Informationsnätverk för cancervården
IR	Incidence rate
IRR	Incidence rate ratio
L1CAM	The L1 neuronal cell-adhesion molecule
LVSI	Lymphovascular space invasion
M	Mitosis
NEC	Non-endometrioid endometrial carcinoma
OR	Odds ratio
OS	Overall survival
P53	Tumor protein p53
PCOS	Polycystic ovary syndrome
PFS	Progression-free survival
PTEN	Phosphatase and tensin homolog
RCC	Regional Cancer Center
RR	Relative risk
RS	Relative survival
S	Synthesis phase
SES	Socioeconomic status

SPF	S-phase fraction
SQRGC	Swedish Quality Registry of Gynecologic Cancer
TP53	Tumor protein p53 gene
UK	United Kingdom
US	The United States of America
VBT	Vaginal brachytherapy
WHO	World Health Organization
WSHCR	Western Swedish Healthcare Region

1 INTRODUCTION

1.1 INCIDENCE AND MORTALITY

1.1.1 INCIDENCE

Endometrial carcinoma (EC) is the 6th most common malignancy in women worldwide with 3821000 new cases 2018 (1). Age-standardized incidence rates (all ages) are highest in Europe and north America, 19 per 100 000, and lowest in middle-income countries like South Africa, 1 per 100 000, and India, 3 per 100 000 (2). The incidence is highest among postmenopausal women and rises with age (2). The incidence rates have increased over time in a majority of countries and most rapidly in countries with the lowest rates (2). In average 1398 women were diagnosed each year with endometrial carcinoma in Sweden 2011-2015 (3).The incidence rates have been increasing in Sweden since the middle of the 80s in elderly postmenopausal women (4).
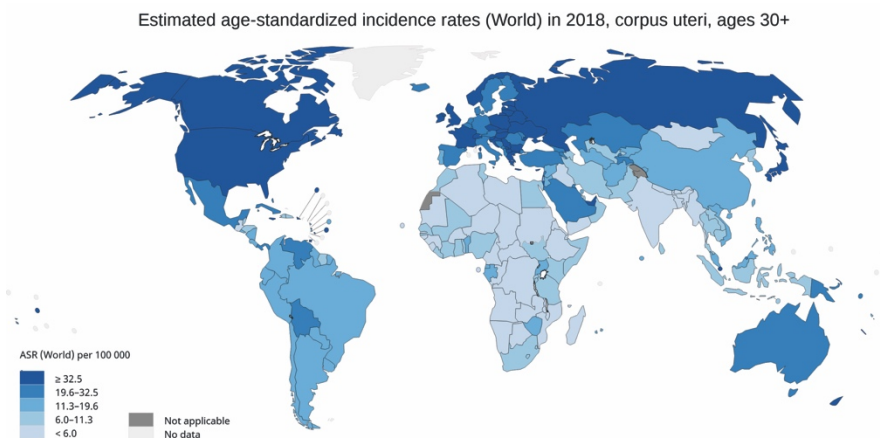
Figure 1. Estimated age-standardized incidence rates of endometrial carcinoma in 2018. GLOBECAN. Published in 2018.

1.1.2 MORTALITY

About 90 000 deaths in EC occured in 2018 (1) In Sweden age-standardized mortality rate is 1.1 per 100 000 with in average 167 deaths/year between 2011-2015 (3). The 1- and 5-year relative survival (RS) is 95% and 85%. Women aged 30-49 and 50-74 at diagnosis have the same RS, about 88-89%, while the oldest women, 75-89 years, have a lower RS at 74% after 5 years (5).
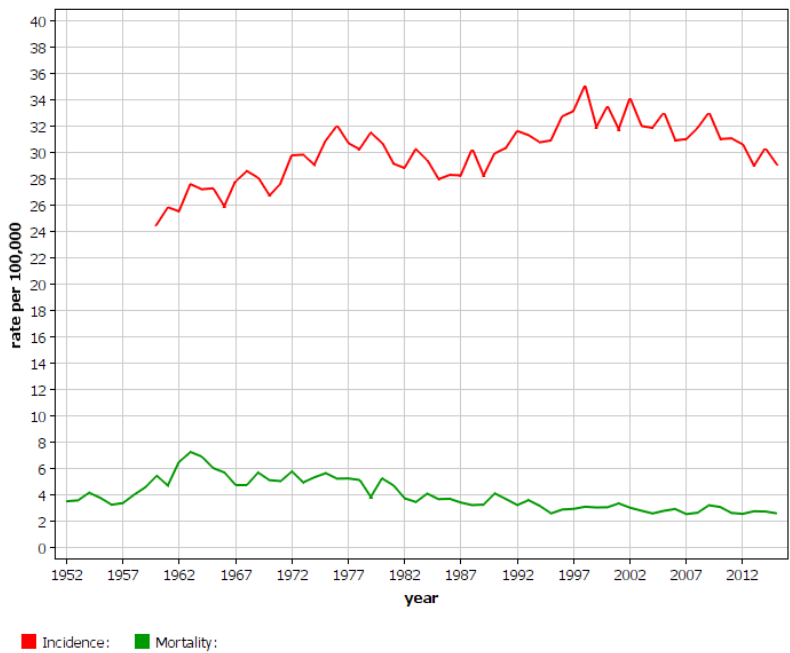
NORDCAN © Association of the Nordic Cancer Registries (22.2.2019)

Figure 2. Estimated age-standardized rates (world) of endometrial carcinoma age 30-85+. NORDCAN, 2019.

1.2 ETIOLOGY

Endometrial cancer originates from the endometrium lining the body of the uterus, in contrast to uterine sarcoma which originate from connective tissue or muscular layer. There are two different pathogenetic types of endometrial cancer; type I and II [4]. Type I, represents 70-80% of EC. It is estrogen related and may arise from complex atypical hyperplasia (6). Type I are characterized by endometrioid (EEC) histology (7) highly or moderately differentiated, a superficial invasion of the myometrium and is associated with good prognosis (6) since they are diagnosed in early stages. Type II are not estrogen dependent and arises in an atrophied endometrium (6). It is characterized by a non-endometrioid (NEC) histology (serous, clear cell, carcinosarcoma, poorly differentiated and undifferentiated carcinoma)(7) with poorly differentiation, deep invasion of the myometrium and is associated with an unfavorable prognosis(6, 8) since they are diagnosed in later stages and are more aggressive. In 1994, WHO classified (WHO94), hyperplasia into 4 subgroups; simple, complex, simple atypical and complex atypical hyperplasia, with marked risk of progression to carcinoma if atypical hyperplasia is present (9). Since 2014, WHO classifies hyperplasia into 2 groups; hyperplasia without atypia and atypical hyperplasia/endometrioid intraepithelial neoplasia.

1.2.1 TYPE I AND TYPE II ENDOMETRIAL CANCER

Type I and type II tumors differ in morphological, clinical and genetical features. The development of EEC type I and NEC type II involves different molecular alterations (10). EEC develop through a premalignant phase of intraepithelial neoplasia in most cases (9) while serous and clear cell arises as a result of genetic mutations (11). EEC is characterized by mutations in PTEN (phosphatase and tensin homolog), KRAS, β-catenin-gene and microsatellite instability (12). PTEN, a tumorsupressor gene, encodes a protein that causes cell cycle arrest at the G1/S-checkpoint and upregulation of proapoptotic

mechanisms. PTEN function could be altered by mutations leading to aberrant cell growth and apoptotic escape. PTEN mutations are identified in up to 80% of EEC and 55% of atypical endometrial hyperplasia, which suggests that it is an early event in endometrial tumorigenesis (13, 14). The RAS-RAF-MEK-ERK signaling pathway play an important role in tumorigenesis and inhibition of growth signals (10). In EEC, most mutations affecting the RAS-RAF-MEK-ERK signaling pathway are found in KRAS (13). KRAS mutations persist in 26 % of all type I EC (15). In mouse models, mutations in KRAS is not sufficient to induce endometrial carcinogens compared to PTEN for example (13) but alterations in KRAS contributes to neoplastic transformation of the endometrium in presence of other alterations. CTNNB1, the β-catenin gene, is an oncogene. Mutations in CTNNB1 results in stabilization of the β-catenin protein with resistance of degradation, accumulation and in complex with DNA binding proteins participate in transcriptional activity (12). β-catenin is a component of the E-cadherin-catenin unit which regulates cell differentiation and tissue architecture. β-catenin is considered an early event in the tumorigenesis since it is present in atypical hyperplasia. Microsatellite instability is demonstrated in 30% of EEC and in 75% of hereditary non-polyposis colon cancer (HNPCC) associated EC (10). Microsatellites are short segments of repetitive DNA bases in both coding and non-coding DNA sequences. Microsatellite instability refers to the propensity to develop alterations in the number of repeated elements in the microsatellites. Mismatch repair deficiencies, lead to accumulation of mutations in DNA sequences including microsatellites. Microsatellite instability is suggested to be an early event in EC (16).

Type II EC is characterized by TP53 mutations, reduced expression of E-cadherin, overexpression of HER-2/neu, alterations in genes controlling the mitotic spindle checkpoint and loss of heterozygosity reflecting chromosomal instability (10). TP53 mutations is demonstrated in 90% of NEC and in 10-

20% of EEC, mostly in grade 3. TP53, a tumor suppressor gene, encodes p53 which promotes cell cycle arrest in G1, and apoptosis when DNA damage is present (12). Mutation in the p53 gene causes accumulation of a nonfunctional protein in the cell. Reduced expression of E-cadherin is present in 80-90% of type II EC (10). It is a transmembrane protein with an intracellular domain connected with the cytoskeleton and reduced expression is associated with cell to cell contact (12). HER-2/neu, an oncogene, encodes a transmembrane receptor involved in cell signaling.

The type I and II classification is rigid since there is an overlap between the two groups and for high-grade EEC classification could be challenging with high intraobservation in variability in histotype diagnosis (7). In 2013, The Cancer Genome Atlas Research Network presented an integrated genomic characterization of EC(17), which will be addressed in the discussion section.

1.3   RISK FACTORS

The endometrium is altered during the menstrual cycle due to fluctuation in estrogen and progesterone, where estrogen induces glandular differentiation and decidualization of the endometrium which is encountered by progesterone where estrogen, a growth factor, promotes growth of endometrial cancer cells in a genomic and non-genomic manner. Estrogen binds to the estrogen receptor (ER) and as a steroid hormone receptor binds to the genome and regulate transcription. In the non-genomic manner, ER, as a cell surface receptor, activates pathways (MAPK, that are involved in the RAS-RAF-MEK-ERK pathway) when binding to estrogen(18) . Estrogen is primarily produced by the ovaries in premenopausal women. In postmenopausal women, peripheral tissue, including adipose tissue, converts androgens to estrone and estradiol by aromatase, an enzyme produced in mesenchymal stromal cells, including adipocyte stem cells and to a lesser extent mature adipocyte. Peripheral tissue is the primary source of estrogen in postmenopausal women (19) .

1.3.1   HIGH BODY MASS INDEX

The prevalence of overweight, defined as BMI 25-30 kg/m^2, and obesity, defined as ≥30 kg/m^2, is increasing globally in women from 29.8% to 38% between 1980 and 2013 (20). Several studies have confirmed that greater body fatness, measured by BMI, increases the risk of EC. The Million Women Study from the UK found an adjusted relative risk of 2.89 per every 10 unit increase in BMI (21). In a meta-analysis including 3132 cancer cases, published 2014, the relative risk of EC increases with increasing BMI (21). A case-control study found that women with continually overweight between 20 and 50 year of age had an almost five folded odd ratio for endometrial cancer risk compared to women who maintained a normal weight. There was a gradient towards higher endometrial cancer risk the longer the overweight consisted, and becoming overweight after 50 year of age increased the risk two folded, three folded after 40 year of age and four folded after 30 year of age (22).

1.3.2   DIABETES MELLITUS

Obesity is closely associated with insulin resistance and hyperglycemia. Several studies support diabetes mellitus as an independent risk factor for endometrial cancer with almost doubled risk of EC if diabetes mellitus is present (23, 24). A meta-analysis supported the independent risk of diabetes mellitus for increased EC risk (25). In type II diabetes mellitus, the association could be confounded by high BMI, which is common among individuals with type II diabetes mellitus (26). A meta-analysis did not find any significant association between metformin and lower risk of EC but improved overall survival (OS) in EC, HR 0.61 (95% CI 0.48-0.77), and reduced risk of recurrence, OR 0.50 (95% CI 0.28-0.92) (27).

1.3.3   TAMOXIFEN

Tamoxifen, used by women with estrogen receptor positive breast cancer, significantly reduces recurrence rate and mortality in breast cancer (28). As a side effect Tamoxifen increases the relative risk of endometrial cancer with 2.53 times (95% CI 1.35- 4.97) (29). The risk is markedly higher in postmenopausal women, RR 4.01 (95% CI 1.70-10.90)(29). The dose and duration of therapy is of importance (30). The histopathology and tumor stages are more aggressive and advanced in long-term use of tamoxifen (31).

1.3.4   UNOPPOSED ESTROGEN

Type I EC is promoted by unopposed estrogens. Anovulation leads to lack of corpus luteum production of progesterone and leads to unopposed growth of the endometrium (18). Polycystic ovary syndrome (PCOS) is a common endocrine illness in reproductive women, with anovulation and unopposed estrogens. A meta-analysis published in 2014 analyzed the risk of EC in women with PCOS, and found an OR at 4.05 (95% CI 2.42-6.76) in premenopausal women(32). Unopposed estrogen as therapy by postmenopausal women without hysterectomy increases the risk of EC 5 folded and 10- to 30-folded with extended treatment more than five years (33) due to the association of endometrial hyperplasia (33).

1.3.5   ETHNICITY

The age-adjusted incidence rate of EC in non-Hispanic black women, Hispanic women and Asian women is lower compared to non-Hispanic white women, RR 0.81 (95% CI 0.80-0.82), RR 0.73 (95% CI 0.71-0.74), RR 0.70 (95% CI 0.68-0.72) (34). Non-Hispanic black women have significantly higher incidence rates of EEC high-grade, carcinosarcoma, serous and clear cell adenocarcinoma compared to non-Hispanic women. Mortality rate is also higher among non-Hispanic black women compared to non-Hispanic white

women, RR 1.55 (95% CI 1.50-1.61). A study from the US demonstrated that socioeconomic, clinicopathological and treatment differed between black and white women (35). Black women were more likely to live in neighborhoods with low-income and low educational attainment compared to white women, fewer black women had localized disease, low grade and type I histology and finally they were less likely to undergo surgery, to have a total hysterectomy but more likely to receive radiation. Black ethnicity was associated with increased all-cause mortality, HR 1.29 (95% CI 1.24-1.34) and cancer specific mortality, HR 1.18 (1.11-1.26) when adjusting for demographics, clinicopathological factors and treatment.

1.3.6 SOCIOECONOMIC FACTORS

There are several indicators of socioeconomic status (SES); level of education, occupational social class and income (36). Level of education provides knowledge, occupational provide relation and network and income provide resources to consume a healthy lifestyle, which all facilitate healthy behavior. In Europe smoking and obesity is more prevalent in individuals with lower level of education (37). A Danish study did not find any association between incidence rate for corpus cancer in basic education compared to higher education, adjusted IRR 0.98, or in low income compared to middle income, adjusted IRR 0.94 (38) but excess mortality was higher among women with basic education during the first 2 years after diagnosis (39).

1.4 TUMOR CHARACTERISTICS

1.4.1 HISTOPATHOLOGY

Endometrial carcinoma is classified according to WHO/International Society of Gynecological Pathology classification (40):

- Endometrioid carcinoma: adenocarcinoma, adenocarcinoma-variants with squamous differentiation, secretory variant, villoglandular variant and ciliated cell variant.

- Mucinous adenocarcinoma

- Serous adenocarcinoma

- Clear cell adenocarcinoma

- Undifferentiated carcinoma

- Neuroendocrine tumors

- Mixed carcinoma (composed of more than one type with ≥ 10% of each component).

Mixed epithelial and mesenchymal tumors:

- Adenomyoma

- Atypical polypoid adenomyoma

- Adenofibroma

- Adenosarcoma

- Carcinoma (treated as aggressive carcinoma)

1.4.2   STAGE ACCORDING TO FIGO

Surgical stage of endometrial carcinoma is classified according to International Federation of Gynecology and Obstetrics (FIGO). In 1988 clinical staging was replaced by surgical stage since 25% of clinical stage 1 were not confined to the uterus. Surgical stage is more precise and clinical stage is now only used in patients who do not go through surgery. The revised 2009 FIGO staging system for endometrial cancer included many changes over the 1988 system, particularly for stage I subgroups; stage I was divided into 2 sub-stages, instead of 3, and stage II changed to 1 sub-stage (41). Stage according to FIGO 2009 were implemented from January 2010 in the Western Swedish Health Care Region (WSHCR). Positive cytology does not change stage but is reported.

Surgical stage according to:

FIGO 1988	FIGO 2009
IA Tumor limited to the endometrium	IA Tumor invasion <50% of the myometrium
IB Tumor invasion to l <50% of the myometrium	IB Tumor invasion ≥ 50% of the myometrium
IC Tumor invasion to ≥ 50% of the myometrium	
IIA Tumor invasion of endocervial glands	II Tumor invasion of cervical stroma
IIB Tumor invasion of cervical stroma	
IIIA Tumor invasion of the serosa and/or adnexae and/or positive cytology	IIIA Tumor invasion of the serosa and/or adnexae
IIIB Metastases in vagina	IIIB Mestases in vagina and/or parametrial involvement
IIIC Metasases to pelvic and/or para-aortic lymph nodes	IIIC1 Metastases to pelvic lymph nodes
	IIIC2 Metastases to para-aortic lymph nodes
IVA Tumor invasion of bladder and/or bowel mucosa	IVA Tumor invasion of bladder and/or bower mucosa
IVB Distant metastases	IVB Distant metastases

Table 1. Surgical stage according to FIGO 1988 and 2009.

1.4.3 HISTOPATHOLOGIC GRADE

Histopathologic degree of differentiation of endometrioid and mucinous adenocarcinomas are classified according to FIGO(40):

- Grade 1 (G1): well differentiated with less than 5% of nonsquamous or nonmorular solid growth.

- Grade 2 (G2): moderately differentiated with 6-50% of nonsquamous or nonmorular solid growth.

- Grade 3 (G3): greater than 50% of nonsquamous or nonmorular solid growth pattern

Nuclear atypia like pleomorphism and prominent nucleoli, raises the grade by 1. Serous and clear cell carcinomas are not graded since they are high risk by definition.

1.5 PROGNOSTIC FACTORS AND RISK GROUPS

Endometrial carcinoma is divided into type I and II. Type I is characterized by low stage, grade 1-2, endometrioid histology and a favorable prognosis. Type II is characterized by higher age, high stage, grade 3, non-endometrioid histology and a poor prognosis. This division is suboptimal and there is a phenotypic overlap since 20% of type I recur and 50% of type II do not (42).

A prognostic factor predicts the natural disease course and provides information of prognosis after standard treatment. This should not be confused with a predictive factor for response to treatment, which are used to evaluate new therapies. The majority of women with EC are older, and often with comorbidity. This entails that there is a need for an individualized and tailored surgery procedure and adjuvant treatment to avoid over- and under treatment.

1.5.1 SURGICAL STAGE AND MYOMETRIAL INVASION

FIGO surgical stage is the strongest prognostic factor in endometrial carcinoma with a disease-specific survival of 96% in stage IA, 87% in stage IB, 80% in stage II, 60% in stage IIIC1, 53% in stage IIIC2 and 16% in stage IVA (43). Since 1988, EC is surgically staged and in 2009 FIGO stage was revised (41). A prospective multicenter trial with a high proportion of patients with lymphadenectomy showed that surgical stage according to FIGO 2009 did not worsening the prognosis for stage I and II, i.e down stage stage IB to stage IA and stage IIA to stage IA or IB (43) and that the revised FIGO stage improved prediction of prognosis. Myometrial invasion separates stage IA from stage IB, thereby a part of the stage classification. In patients without lymphadenectomy, with uncertain nodal status, myometrial invasion is a significant prognostic factor (p=0.001) but the prognostic effect of myometrial

invasion was not present in patients with lymphadenectomy (p=0.205) (43). In multivariable analysis myometrial invasion is an independent prognostic factor for survival with adjusted HR 3.91 (95% CI 1.35-11.36, p=0.012) (43) and a significant prognostic factor for lymph node metastasis (RR 4.10, 95% CI 2.99-5.61)(44).

1.5.2 HISTOPATHOLOGY

EEC is the most common histotype, 84%, serous adenocarcinoma and clear cell adenocarcinoma represent only 6-10% of EC but accounts for more than 50% of recurrences (45, 46). The OS at 5-year is 83% for EEC histotype, 62% for clear cell and 53% for serous adenocarcinoma (45). The 5-year OS in stage I EEC is 90% compared to 85% for clear cell and 80% for serous adenocarcinoma (45). The 5-year OS for carcinosarcoma (malignant mixed Mullerian tumor) is 30% and in stage I about 50% (47) and has a significantly worse outcome compared to non-endometrioid carcinoma, HR 3.1 (95% CI, 1.5-6.8).

1.5.3 HISTOPATHOLOGIC GRADE

Most endometrioid carcinomas are grade 1 and 2. The amount of positive pelvic and para-aortic lymph nodes increases with increased grade (45), and of patients with both G3 and deep myometrial invasion 37% have pelvic node metastases and 13% have para-aortic node metastases. The 5-year OS in stage I G1 is 93%, in stage I G2 90% and in stage I G3 79% and an adjusted HR in stage I of 1.4 (95% CI 1.1-1.7) for G2 and 2.8 (95% CI 2.2-3.6) for G3 (45). In endometroid carcinoma grade is an independent prognostic factor for cause-specific death, HR 2.7 and 7.7, for G2 and G3 (p=0.003) (48).

Grade 3 EEC, serous and clear cell adenocarcinoma are classified as type II. EEC grade 3, have a 5-year disease-specific survival (DSS) of 77%, compared to serous, 55%, and clear cell, 68% (49). Serous and clear cell carcinomas also

have significantly more advanced stages compared to G3 EEC, still these trends remain when stratified for stage (49).

1.5.4 AGE

Survival in EC is decreasing with increased age, with 5-year RS of 70% in the age group 80-89-year-old, 82% in 70-79 and 87% in 60-69 and above 90% in age <60-year-old (50). Age is an independent prognostic factor for survival in EC although various cut-off's have been used. The PORTEC-1 study used age 60 as cut-off and GOG-99 ≤50, 50-69 and ≥70 (51, 52). In EC multivariate analysis, age ≥70 year was associated with impaired survival with HR 1.634 (95% CI 1.248-2.138) (53) and in stage I EC the risk of endometrial-cancer-related death was increased for patients with age ≥60 year (HR 3.1, p=0.02)(51).

1.5.5 LYMPHOVASCULAR SPACE INVASION

Presence of tumor cells in the lymphatic or vascular spaces of the uterus (LVSI) in EC is found in 25% (54). Creasman et al reported capillary-like space involvement in 1987 when studying the pathological spread patterns of EC (55) and demonstrated an association with positive pelvic and para-aortic lymph nodes. Several studies find LVSI to be an independent prognostic factor for lymph node metastases and relapse (56, 57). In EEC LVSI is associated with decreased over-all survival (HR 2.04, 95%CI 1.49-2.79) and decreased progression-free survival (HR 2.19, 95% CI 1.62-2.96) (58). Vascular invasion was registered in the WSHCR from 2006, but it is not used clinically in Sweden yet because of difficulties of defining LVSI.

1.5.6 DNA PLOIDY

Aneuploidy occurs as a result of mitotic errors from chromosomal rearrangements and is the presence of abnormal number of chromosomes in

the cells (59). DNA ploidy is more frequent in serous and clear cell carcinoma, 29-52% than in EEC, 23-25% (60, 61). DNA ploidy is analyzed in both fresh frozen and paraffin embedded tissue. Analyses on fresh frozen tissue permits detection of hypoploidy. DNA ploidy is analyzed by flow cytometry or image cytometry, a more sensitive method, since analysis determination is available for as few as 100 cells compared to flow cytometry which requires more than 5000 cells (62).

DNA ploidy have an independent prognostic impact in stage I-IV EC in some but not all studies using multivariate analyses including histologic subtype (63). These inconclusive results may be explained by different cut-off points for the DNA index, sample quality and potential confounders included in the survival analyses (63). In prior studies in FIGO stage I-II EEC, DNA ploidy had no independent prognostic impact (64, 65). A nationwide population based Swedish study did not find DNA ploidy to be a prognostic factor for lymph node metastasis (RR 1.15, 95% CI 0.86-1.53)(44).

1.5.7   P53

Mutations in the TP53 gene is present in 93-100% of serous adenocarcinoma and in 17-61% in EEC (13). The TP53 gene is involved in regulation of the G1 cell cycle arrest, when DNA damage has occurred, and in apoptosis. Errors in the cell cycle regulation leads to uncontrolled growth. Studies on endometrial carcinoma have demonstrated an independent prognostic impact of p53 on survival, with HR 4.9 (95% CI 1.3-17.6)(66) on hysterectomy specimens and with an adjusted HR at 2.8 (95% CI 1.5-5.4) on curettage(67). Univariate analyses on curettage of endometrioid tumor FIGO stage I demonstrated a significant survival decrease in p53 positive tumors (p<0.001)(67). The prognostic effect of p53 overexpression is not always independent of histological subtype (42), but in a study on curettage , FIGO stage I–II EEC, p53 had an independent prognostic impact in a multivariate analysis (68). Observational studies also support p53 overexpression as a prognostic factor

for extra uterine disease with lymph node dissemination in endometrial carcinoma (69) in both endometrioid and non-endometrioid EC (70).
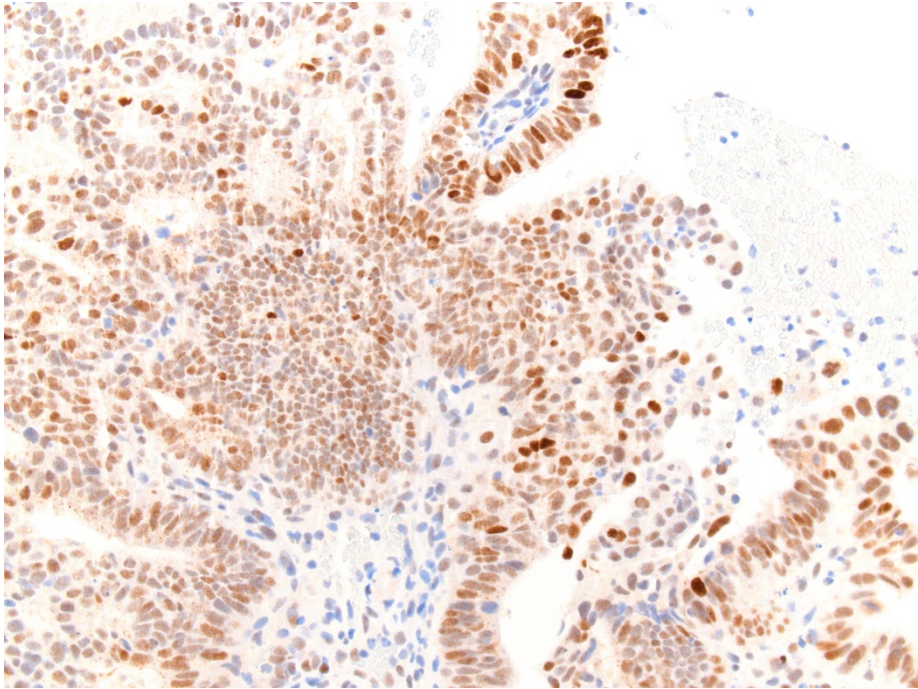
Figure 3. Immunohistochemistry staining of p53 wild type.

1.5.8   S-PHASE FRACTION

The cell cycle consists of four phases, G1, S, G2 and M. In the s-phase the DNA synthesis occur and the genetic material is duplicated. S-phase fraction (SPF), the proportion of tumor cells that are in the s-phase, is a marker of proliferative activity. In 1985 Tsou et al demonstrated significant differences between normal and cancer endometrium specimens, with an average DNA distribution of 14.3% in S-phase compared to 8.4% in the cancer and non-cancer specimens (71). SPF is associated with histological grade, histologic

subtype, stage and age (72-74). Studies of SPF by flow cytometry have shown prognostic impact on survival in EC and EEC (73, 74). In stage I EEC, however the results are conflicting (75), although a large recent Swedish study on SPF found it to be an independent prognostic factor when adjusted for stage, age and grade (65).

1.5.9 RISK GROUP CLASSIFICATION

Patients with EC have been categorized into risk groups with two main purposes; 1) to identify patients with lymph node involvement preoperative, which are in need of referral to specialized units, lymphadenectomy and spared extensive surgery 2) to identifying patients with risk of recurrence that would benefit from adjuvant therapy, based on assessment of hysterectomy specimens and categorized into postoperative risk groups. There has been no consensus on risk group classification internationally and various prognostic factors have been included in each classification. As an example, the PORTEC-1 study and GOG-99 study used different classifications. The PORTEC-1 study (2000) (51), divided patients into four risk groups;

- Low risk: EEC stage Ia, G1
- Intermediate: EEC, stage I with G1/myometrial invasion ≥50% or G2 or G3/myometrial invasion <50%
- High-intermediate: Age >60 years with G1-2/myometrial invasion >50% or age >60 years with G3/myometrial invasion <50%
- High risk: stage III-IV or serous carcinoma or clear cell carcinoma of any stage

The GOG-99 study (2004) (52), divided patients into four risk groups and defined risk factors as G2 or G3, LVSI, myometrial invasion to outer 1/3;

- Low risk: EEC stage Ia, G1-2

- Low-intermediate risk: age ≤50 years/≤ 2 risk factors or age 50-69 years/≤1 risk factor or age ≥70 years/no risk factor

- High-intermediate risk: 3 risk factors or age 50-69 years/≥risk factors or age ≥70 years/≥ 1 risk factor

- High-risk: stage III-IV or serous carcinoma or clear cell carcinoma of any stage

When preoperative investigation has excluded extrauterine spread, risk assessment is based on the histopathologic examination of endometrial biopsy or curettage. The primary surgical strategy is based on high risk features even if there could be considerable discrepancies between preoperative evaluation and histopathology assessment of hysterectomy specimens mostly for endometrioid tumors (76). Lymphadenectomy is performed primarily for accurate staging in patients with high-risk of lymph node involvement (40). Lymphadenectomy of patients with almost no risk of extra uterine disease will only increase the risk of complications in contrast to patients with high risk of extra uterine disease where lymph node positive patient, surgical stage IIIC, are in need of adjuvant treatment (40, 77). The ESMO-ESGO-ESTRO consensus conference on endometrial cancer published 2016, concluded that low-risk EEC (grade 1 or 2, myometrial invasion <50%), who have low risk of lymph node involvement, should not be recommended lymphadenectomy (78), while patients with intermediate risk (myometrial invasion >50% or grade 3) could be considered for lymphadenectomy for accurate staging (78). High-risk patients (grade 3 and myometrial invasion >50%) should be recommended lymphadenectomy (78).

Based on results of trials evaluating adjuvant radiotherapy(51, 52, 79, 80) the ESMO-ESGO-ESTRO consensus conference (2016) on endometrial cancer defined risk groups in stage I EEC(78):

- Low: G1-2, myometrial invasion <50%, LVSI negative

- Intermediate: G1-2, myometrial invasion ≥50%, LVSI negative

- High intermediate: G3, myometrial invasion <50%, regardless of LVSI status, or G1-2, LVSI positive regardless of depth of invasion

- High: G3, myometrial invasion ≥50%, regardless of LVSI status.

Non-endometrioid carcinoma including serous, clear-cell, undifferentiated and carcinosarcoma are classified as high-risk(78). The FIGO cancer report from 2018 suggests tumor grade 3, LVSI, non-endometrioid histology (including serous, clear cell and undifferentiated) and cervical stromal involvement (surgical stage II) determine high-risk patients (40). New genomic subgroups based on the Cancer Genome Atlas was suggested in 2013 (17) and tested for its prognostic relevance in the PORTEC and ProMise cohorts (81, 82). All subgroups have a distinct prognosis (17). This will be further discussed in the discussion.

1.6   TREATMENT

1.6.1   SURGERY

Surgery is the cornerstone of EC treatment. 1988 clinical stage was replaced by surgical stage. Surgical staging includes vertical midline incision, peritoneal washing, exploration of the intra-abdominal contents, with palpation of the diaphragm, liver, omentum, intestines, peritoneum and adnexal structures (40). There after palpation of pelvic and para-aortic suspicious or enlarged lymph

nodes. Hysterectomy and bilateral salpingo-oophorectomy are the standard procedure. Laparoscopic surgery is recommended in early stages and is proven to result in equivalent recurrence rate, over-all survival and disease-free survival compared to laparotomy(83, 84).

1.6.2 LYMPHADENECTOMY

Lymphadenectomy is required for complete staging. There is no standardized definition of adequate lymphadenectomy(78). According to ESMO-ESGO-ESTRO consensus conference the current approaches include pelvic and para-aortic lymphadenectomy to the inferior mesenteric artery and up to the renal vessels. (78). More than 10 pelvic lymph nodes should be removed for an accurate lymphadenectomy(85). Lymphadenectomy provides a more correct estimation of prognosis and triage of adjuvant therapy. Low-risk group (grade 1-2, myometrial invasion <50%) have low prevalence of lymph node involvement, 1.4%, compared to high-risk group 6.4%(86). The therapeutic effect of lymphadenectomy is controversial. Two randomized trials evaluating pelvic lymphadenectomy in stage I did not find any differences in over-all, recurrence-free or disease-specific survival in stage I with pelvic lymphadenectomy compared to standard surgery(87, 88). Lymphadenectomy is recommended in high-risk (grade 3, myometrial invasion ≥50% or non-endometrioid) and could be considered in intermediate-risk for staging purpose and is not recommended in low-risk(78).

1.6.3 ADJUVANT TREATMENT

Indication for adjuvant therapy is based on risk-group consisting of risk-factors for recurrence. Adjuvant treatment, radiotherapy, chemotherapy or a combination, is not risk free. They are associated with both acute and delayed toxic effect on EC patients whom the majority is older and often comorbid (89, 90). Low-risk endometrial carcinoma (stage I, grade 1-2, myometrial invasion <50%) does not benefit from adjuvant radiotherapy (91) with a 96% 5-year

survival with surgery alone (40). Adjuvant treatment is not recommended in low-risk EC is and is treated with surgery alone (78).

1.6.4   RADIOTHERAPY

The role of pelvic external beam radiotherapy (EBRT) in early stage EC was studied in three randomized studies (51, 52, 79). In all these studies intermediate-risk patients were randomized after total hysterectomy and bilateral salpingo-oophorectomy to pelvic EBRT or observation. There was no overall survival benefit with EBRT but significant lower frequency of locoregional recurrence for intermediate-risk EC. Vaginal brachytherapy (VBT) was compared with pelvic EBRT in the randomized PORTEC-2 trial (92) on high-intermediate risk-group with equivalent low numbers of vaginal recurrence (1.6% for EBRT vs 1.8% VBT) in both arms. The high-risk group consists of both EEC with high-risk features and non-endometrioid endometrial carcinoma. High-risk patients with grade 3 and myometrial invasion $\geq$50% have increased risk of pelvic recurrence and distant metastases together with an impaired overall survival at 58% (93). EBRT is the standard treatment for high-risk patients (40).

1.6.5   CHEMOTHERAPY

Chemotherapy is used in both early stages, in patients with increased risk of micro metastases, and advanced stages of EC. Randomized studies with adjuvant ERBT vs chemotherapy have found similar impact on progression-free and overall survival(94, 95). Combination of ERBT and chemotherapy in EC was studied in a meta-analysis where progression-free survival (PFS) was improved in the ERBT plus chemotherapy arm, 78% vs 69% (p=0.009) (90). A Cochrane review, published in 2011, found that adjuvant chemotherapy improved OS, HR 0.74 (95% CI 0.62-0.92), PFS, HR 0.75 (95% CI 0.64-0.89) and reduced risk of distant recurrences outside pelvis, RR0.79 (95% CI 0.68-

0.92)(89). When analyzing trials with high dose platinum regimens, adjuvant therapy was associated with an absolute risk reduction of death with 4%.

2 AIM

The over-all aims of this thesis were to evaluate the associations between prognostic factors and excess mortality, between socioeconomic and immigrant status and incidence rate.

The specific aims were:

- To evaluate the association of protocol changes on excess mortality, among patients with endometrioid and non-endometrioid endometrial adenocarcinoma and to evaluate associations between age and excess mortality. A secondary aim was to examine differences in treatment administered in the two cohorts (Paper I).

- To analyze the associations of overexpression of p53, elevated s-phase fraction and aneuploidy in endometrioid endometrial carcinoma on excess mortality (Paper II).

- To examine the association between socioeconomic status, defined by educational level, and immigrant status and stage-specific incidence rates of endometrioid endometrial carcinoma and non-endometrioid endometrial carcinoma (Paper III).

3 PATIENTS AND METHODS

3.1 SETTING

Studies included in this thesis were conducted in the WSHCR, a geographic area in Sweden. There are unique possibilities to perform epidemiology studies in Sweden due to the domination of public healthcare, personal identity number, regional and nationwide registers. This implies an almost total population coverage of nationwide and regional registers, and as a result biased selection of study population is limited. The unique Swedish identity numbers enables linkage between population registers(96).

The WSHCR consists of the Västra Götaland Region and the northern part of Region Halland. The population of the WSHCR amounts to 2 million inhabitants, i.e. 20% of the Swedish population. The WSHCR harbor five hospitals; Sahlgrenska University Hospital, Skaraborg Hospital, Södra Älvsborg Hospital, Northern Älvsborg County Hospital and Halland's Hospital Varberg, that provides gynecological care. Adjuvant therapy was decided at The Department of Gynecologic Oncology at the Sahlgrenska University Hospital and managed at all participating hospitals.

3.2 DATA SOURCES

3.2.1 THE SWEDISH CANCER REGISTER

The Swedish Cancer Register is held by the National Board of Health and Welfare. Its primary purpose is to register and monitor cancer incidence and survival. It was founded in 1958 and notification of malignant and some benign tumors are obligatory for health care providers, thereby covering the whole population. For every cancer diagnosed a report has to be sent to the regional cancer registries at regional cancer centers situated in each health care

region in Sweden. The regional registries encode and register data. Annually new cases are reported to the Swedish Cancer Register at the National Board of Health and Welfare. The register contains data on the patient (personal identification number, sex, age, place of residence) medical data (tumor site, histological type, stage according to FIGO, basis of diagnosis, reporting hospital and department, reporting pathology/cytology department) and follow-up data (date and cause of death, date of migration) (97). Date and cause of death is provided from the cause of death register. Data on migration and residency in Sweden is provided from the Statistics Sweden Population Register. The quality of data is maintained by checking identification number against the register covering the total population of Sweden, of duplicates, the validity and logical contents of the codes. The Swedish Cancer Register has an estimated underreporting rate at 3.7% in 1998 (98) and 3.4% for female genital organs. The degree of underreporting varied by diagnostic group (all cancers) and age(98) . The register was used in all articles included in this thesis.

3.2.2   WESTERN SWEDISH HEALTH CARE REGION CLINICAL REGISTRY FOR ENDOMETRIAL CANCER

The WSHCR Clinical Registry for EC was founded in 1995 and held by the Regional Cancer Center (RCC) West. It was introduced along with a complex clinical protocol guiding risk group classification, surgery and adjuvant therapy in EC. In September 2006 the clinical protocol was changed, to a more individualized risk group classification. Lymphadenectomy was performed in selected patients with node negative patients not recommended EBRT.

All data were reported to the registry prospectively by gynecologist, pathologist and oncologist responsible for the individual health care. Data were also reported post-surgery, after completed adjuvant therapy, and annually during follow-up. The registry contains data on the patient (personal

identification number, sex and age) and medical data (reporting gynecologist, hospital and department, date of dilation and curettage, clinical and surgical stage, basis of diagnose, surgery, surgical experience, intention of surgery, lymphadenectomy, pathological lymph nodes, histopathology, adjuvant therapy, grade, myometrial invasion, peritoneal cytology, vascular invasion, DNA–index, SPF, p53 IHC, p53 mutation analysis, risk group) and follow-up (tumor status annually, date and site of recurrence, date and cause of death, autopsy). The Clinical Register for EC was in use between January 1995-December 2009. Participation in the register is optional. There is no available validation of the WSHCR Clinical Register for EC. Data from the register was used in article I, II and III.

3.2.3   THE SWEDISH CANCER QUALITY REGISTRY OF GYNECOLOGIC CANCER (SQRGC)

The objective of "informationsnätverk för cancervården" (INCA) a national IT platform for cancer quality registries, is to provide improvement and quality assurance of gynecological oncology health care (99). Another purpose is to provide data for registration in FIGO for international comparisons. A meeting with leading gynecologic oncologist in Sweden in 2003 the need of a national quality register for gynecological cancer was identified, and the 1st January 2010 data were reported in INCA. Data are reported via five forms: notification, surgical treatment, completed primary treatment, completed non-surgical recurrence therapy and follow-up. Patients with newly diagnosed endometrial carcinoma, are included, Patients diagnosed at autopsy are not included.

The SQRGC was validated 2017 using medical records from 250 patients with EC, including Sahlgrenska University hospital (100). Variables validated included among others were stage according to FIGO, morphology, grade according to FIGO, DNA ploidy, primary treatment. There was a 100%

coverage compared to the obligatory Swedish Cancer Register in WSHCR. Surgical stage was concordant in 85.37%, grade 72.34%, DNA ploidy 68.09% and 92.86% for primary treatment. The SQRGC is considered certification level 3 (of 4 levels, level 1 considered the highest) by Swedish National Quality Registries at Swedish Association of Local Authorities and Regions (101). Data from the SQRGC was used in article I, II and III.

3.2.4 STATISTICS SWEDEN POPULATION REGISTRY

The register is held by Statistics Sweden since 1968. The register is based on the national registration database at The Swedish Tax Agency. Variables registered in the Population Registry among others are personal identification number, sex, age, foreign background, birth country group, vital status (deceased, emigrated). Every night data on death and emigration were transferred to the SQRGC from the Swedish Population Registry at The Swedish Tax Agency. Data from the Swedish Population Register was used in article I and II. Data from the Statistics Sweden Population Registry was used in article III.

3.2.5 THE SWEDISH REGISTER OF EDUCATION

The Swedish Register of Education is held by the National Board of Health and Welfare and its first version concerned level of education from December 1985. The register is updated annually with data provided by The Swedish Public Employment Service, The National Board of Health and Welfare, The Swedish National Agency for Higher Vocational Education and The Swedish Council for Higher Education. Sex, age, country of birth, residency of municipality and county are provided from Statistics Sweden Population Register. Educational level among immigrant originates primarily from questionnaire surveys from newly immigrated individuals and from population

and housing census. The register contains following variables: higher individual level of education, educational year (varying coverage), educational location (varying coverage).

The Swedish education nomenclature has varied over time. In article III we used number of school years completed at the end of the year of diagnose [low≤9 years (primary school), intermediate 10-12 years (high school/pre-university level) and high≥13 years (university level)].

3.3 STUDY POPULATION

3.3.1 STUDY I

3.3.2 STUDY POPULATIONS

The study population was based on the WSHCR Clinical Registry for Endometrial Cancer and SQRGC. Women diagnosed with EC between January 1st 1995 and December 31st 2011 and resident in the WSHCR region at diagnose were included in the study. Sarcomas and patients declining participation in the WSHCR Clinical Register for EC and SQRGC were excluded together with patients (<1%) reported to the WSHCR Cancer Register but not to the WSHCR Clinical EC Register.

The EEC group included; adenocarcinoma (snomed 81403) and adenocarcinoma papillary (82602), constituting 3837 (88.5%) patients. The NEC group included; Mullerian mixed tumors (89503), carcinosarcoma (89803), adenosquamous (85603), adenoacanthoma (85703), squamous carcinoma (80703), clear cell (83103), mucinous (84803) and serous (84603). Mullerian mixed tumors and carcinosarcoma were included in the carcinosarcoma group, mucinous carcinoma was included in the clear cell group, others were included in the serous group.

3.3.3 EXPOSURES

Patients were stratified into cohort 1, defined as patients diagnosed between January 1st 1995 and September 10th 2006 and cohort 2 September 11th 2006 and December 31th 2011. Each time period corresponded to a clinical protocol. The clinical protocol used in cohort 1 included:

- FIGO stage according to 1988.

- Stage I was divided into 3 risk-groups:
    - Low-risk: EEC, G1-2, myometrial invasion <50%,
    - Intermediate-risk: EEC and presence of one of following: G3 tumor, myometrial invasion ≥50% or aneuploidy.
    - High-risk: NEC or EEC and presence of two of following: G3, myometrial invasion ≥50% or aneuploidy.

- Surgery was performed by laparotomy. Enlarged lymph nodes were removed but lymphadenectomy was not performed routinely.

- Stage I low-risk was treated with hysterectomy and bilateral salpingo-oophorectomy (BSO).

- Stage I intermediate-risk was treated with additional EBRT and VBT.

- Stage I high-risk and stage II-III was treated with additional chemotherapy with four cycles of cisplatin (Platinol; Bristol-Myers Squibb, Solna, Sweden) and epirubicin (Farmorubicin; Pfizer, Sollentuna, Sweden).

- Patients with FIGO stage IV disease received individualized treatment.

Behandlingsscheman för endometriecancer

Primäropererade patienter. Riskgrupper enligt bilaga 3

KIR. STAD.	RISK	Strålbehandling EXTERN 46 Gy = 5 v	Strålbehandling VAGINAL 3 x 3,75 Gy = 3 v	Beh 1 + 4 på JK-gyn CYTOSTAT. 4 beh. = 12 v

O P E R A T I O N

I	Låg			
I / II	Medel –	3-4 v → X	1 v → X	
I, II	Hög	3-4 v → X	1 v → X	2-3 v → X
III		3-4 v → X	1 v → X	2-3 v → X Ev hormonell
IV		3-4 v → INDIVIDUELL HANDLÄGGNING		
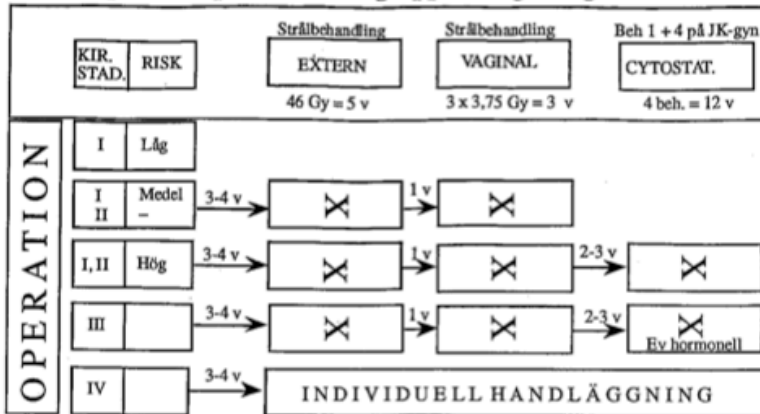
Inoperabla eller ej primärt opererade.

KLIN. STAD	Strålbehandling EXTERN 46 Gy = 5 v	Strålbehandling INTRAUTERIN OCH VAGINAL 3 x 8 + 8 Gy = 6 v	INDIVIDUELL

A B R A S I O

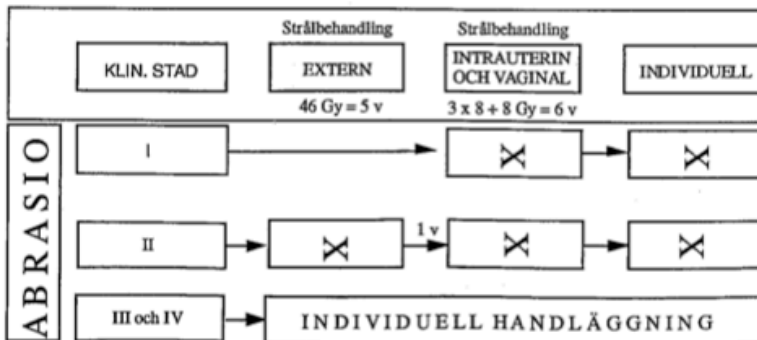| I | → | X | X |
| II | → X | 1 v → X | → X |
| III och IV | → INDIVIDUELL HANDLÄGGNING | | |

Figure 4. Treatment schedule in cohort 1, clinical protocol 1995-2006.

During the time period defined as cohort 2 patients were treated according to the WSHCR clinical protocol used during the same time period. The clinical protocol used in cohort 2 included:

- FIGO stage according to 1988 and 2009 (from 1th January 2010).
- Stage I was divided into 3 risk-groups:
  - Low-risk: as in cohort 1 with SPF >8%, p53 negative tumors.
  - Intermediate-risk:

    A: as low-risk in cohort 1 with SPF >8%, p53 negative.

    B: as intermediate-risk in cohort 1 with age <70 years or p53 positive tumor

    C. as intermediate-risk in cohort 2 with age ≥70 years or p53 positive tumor

  - High-risk: as high-risk in cohort 1.
- Surgery was performed by laparotomy or laparoscopy and included hysterectomy and BSO. Enlarged lymph nodes were removed but lymphadenectomy was only performed routinely in patients with intermediate- risk B or C, and suspected FIGO stage II EC.
- Stage I low-risk was treated with hysterectomy and BSO.
- Stage I intermediate-risk A was treated with additional VBT.
- Stage I intermediate-risk B and C were treated with additional EBRT if lymphadenectomy was not performed. If lymphadenectomy was performed and nodes were negative EBRT was excluded.
- Stage I high-risk and stage II-III were treated with additional chemotherapy with four cycles of paclitaxel (Taxol; Bristol-Myers Squibb) and carboplatin (Paraplatin; Bristol-Myers Squibb). Stage II was treated with lymphadenectomy.
- Stage IV received individualized treatment.

3.3.4   OUTCOMES

The main outcome in article I was RS, with five-year follow-up after diagnose or until death date, in cohort 1 and 2, EEC and NEC analyzed separately. RS was based on date of diagnose data from the WSHCR clinical registry and SQRGC. Date of death data was collected from the Swedish population register. The follow-up was set to five years since our analyses showed stable survival after five years. Secondary outcome was differences in treatment between cohort 1 and 2, EEC and NEC analyzed separately.

3.3.5   VARIABLES

Patient characteristics such as histopathology, FIGO stage, grade, age, surgery, adjuvant therapy was obtained from the WSHCR clinical registry and SQRGC. Date of death was obtained from the Swedish population register.

3.3.6   STUDY II

3.3.7   STUDY POPULATIONS

The study population in article II consisted of women diagnosed with EC stage I-III EEC between September 11th 2006 and December 31th 2011 and resident in the WSHCR. FIGO stage III was included since positive lymph nodes are not always known before surgery. FIGO stage IV, NEC and patients declining participation in the WSHCR clinical registry and SQRGC were excluded.

3.3.8   EXPOSURES

P53, DNA ploidy and SPF were analyzed on tissue collected at either curettage, hysterectomy or both. DNA ploidy and SPF were analyzed with flow cytometry on both formalin-fixed paraffin-embedded (FFPE) and fresh frozen tissue samples. Both flow cytometry and image cytometry are used to measure DNA content. Flow cytometry requires tissue disaggregation and a minimum of 5000-10000 cells compared to image cytometry that is available for as few as 100 cells, therefore considered a more sensitive method compared to flow

cytometry. Flow cytometry measures the content of individual stained cells and the distribution of cells across the cell cycle (102). Number of chromosomes in a germ cell is called the haploid number n, and in a somatic cell it is called the diploid number, 2n. Aneuploidy is when an abnormal number of chromosomes is present in the cell and thereby a change in the DNA amount. The DNA amount can be expressed as a DNA index, the ratio between DNA content of a tumor cell and a normal diploid cell.

The analyze results in a histogram which shows the distribution of cells in different phases of the cell cycle (62). DNA index of 1.0 correspond to a normal number of chromosomes, 2n or 46, of cells in G0 and G1. FFPE tumor biopsies were classified as diploid if there was one G0/G1 peak and fresh frozen tumor biopsies were classified as diploid when DNA index was 1.0 (+/-0.04). The SPF cut off was set to <8% and ≥8%.

Immunohistochemistry (IHC) was used to evaluate the p53 protein expression level. IHC identifies antigens by an antibody specific to the antigen on tissue sections. Both a primary and secondary antibody was used. Background staining of endogenous peroxidase activity was blocked and the avidin-biotin method was used to produce a colored label (103). Both staining intensity and proportion were evaluated, overexpression of p53 was identified when strong nuclear staingin in >30% was present (70).

3.3.9   OUTCOMES

The main outcome in article II was RS, with five years follow-up from date of diagnose to death date, in p53 overexpression compared to p53 negative, aneuploidy compared to diploidy and SPF ≥8% compared to SPF <8%. Relative survival was analyzed separately in p53, DNA ploidy and SPF. Date of death data was collected from the Swedish population registry. The follow-up time was set to five years since our data showed relatively stable survival after five years.

3.3.10   VARIABLES

Patients characteristics such as age, FIGO stage, grade p53 expression, DNA ploidy and SPF were obtained from the WSHCR clinical registry and SQRGC. Date of death were obtained from the Swedish Population Registry.

3.3.11 STUDY III

3.3.12   STUDY POPULATIONS

The study population in article III consisted of women resided in the WSHCR and diagnosed with EC between 1th January 1995 and 31th December 2016. All patients with EC were identified by matching against the Swedish Cancer Register. Clinical data was obtained from patients with informed consent and registered in the WSHCR Clinical Registry for Endometrial Cancer and the SQRGC. Patients declining participation in these registries were excluded. Patient data were linked to The Swedish Register of Education and Statistics Sweden Population Registry using identity numbers (96). Data on immigrant status was restricted to 2000-2016. The cohort in study III was dynamic, which means that individuals could move to or leave the geographic region and therefore add to or leave the cohort.

The EEC group included; adenocarcinoma (81403, 82633) and adenocarcinoma papillary (82602), mucinous (84803, 84703), adenoacanthoma (85703), squamous carcinoma (80703), adenosquamous (85603), carcinoma (80103). The NEC group included; Mullerian mixed tumors (89503), carcinosarcoma (89803), clear cell (83103), mucinous (84803) and serous (84603, 84413), serous intraepithelial carcinoma (84412), neuroendocrine tumor (80413), primitive neuroectodermal tumor (94733) and low differentiated carcinoma (80203).

3.3.13   EXPOSURES

In article III exposure to educational level and immigrant status were examined. Educational level was stratified in relation to the number of school years completed at the end of the year of diagnosis; low ≤9 years (primary school), intermediate 10-12 years (high school/pre-university level) and high ≥ 13 years (university level). Immigrant status was classified as Swedish-/foreign-born based on country of birth.

3.3.14   OUTCOMES

The main outcome in article III was stage-specific incidence of EEC and NEC. Patients were staged according to FIGO 2009. Data on surgical stage was used primarily and clinical stage secondarily if data on surgical stage was missing.

3.3.15   VARIABLES

Clinical data including histopathologic type, surgical and clinical stage were obtained from the WSHCR Clinical Registry for Endometrial Cancer (1995-2009) and the SQRGC (2010-2016). Clinical data were reported by the gynecologist performing surgery. Data on country of birth and educational level were obtained from the Statistics Swedish Population Registry respectively the Swedish Registry of Education. Age were classified into 3 groups; 30-49, 50-74 and ≥75-year-old at diagnose. The lower limit was considered relevant for educational attainment. Only two patients were below 30 years of diagnosis.

3.4   STATISTICAL ANALYSES

Over-all survival: OS measures total mortality. When outcome is death due to any cause, over-all survival represents the proportion of patients alive after a defined time period after diagnosis (104).

Cause-specific survival: Cause-specific survival measures mortality due to a specific cancer and the cause-specific survival rate represents the proportion of patients who did not die of the specific cancer after a defined time-period after diagnosis (104). Cause-specific survival could be skewed due to competing risks (selection bias) and misclassification of cancer-specific cause of death (information bias) (105). A competing risk is death from other causes that precludes death from the specific cancer and therefor death due to non-cancer is censored at the time for death in cancer-specific analysis. Competing risk causes selection bias if subjects censored had different cancer-specific survival than those not censored. Cause-specific survival is dependent on the quality of death data from death certificates and there could be misclassification of cancer-specific deaths and cancer-consequent deaths (105).

Relative survival: RS analyze the survival of a cohort with a specific cancer compared with a cohort without this specific cancer with the same demographic characteristics, usual the general population. The excess mortality rate is the difference between the observed over-all mortality in the cancer cohort and the expected mortality estimated from the comparison group without cancer (105). The cumulative expected survival is calculated by using population life tables, matched for sex, age and calendar year, to estimate the probability of survival in the comparison group. There are three different methods for estimation of expected survival, the Ederer I and II and the Hakulinen methods. RS can be biased by lacking comparability between the cancer group, whom observed survival is calculated, and the comparison group, whom the expected survival is calculated. RS is not biased by competing risks or misclassification of causes of death (105).

Poisson regression analysis: Regression analysis is used for studying the relationship between different variables or to predict prognosis with prediction models. Poisson regression is used for discrete outcome variables, and is sensitive for rates defined as number of events per person per period of time

and number of binary events per population per period of time. Poisson regression analysis is used for estimating incidence rate ratios and excess mortality ratios, since time is included. Poisson regression modeling of excess mortality allows assessment of the effect of multiple variables on excess mortality and survival.

Incidence rate: Incidence rate is the number of new cases in a population divided by the time at risk of disease. The denominator includes all time each person was at risk of getting the event, the so-called person-time. Mortality rate is an incidence rate where the event is death.

Marginal mean: A marginal mean yields the expected outcome for a specific value on an explanatory variable, calculated as the mean of the expected outcomes over the range of the other explanatory variables. For example, the marginal mean may reflect the expected incidence rate for at specific calendar year, calculated as the mean of the expected incidence rates over the age groups 30-34, 35-39, …, 85-89, 90+

Interaction analysis: Statistical interaction is defined as the comparison between the observed and the expected joint effect of an exposure variable and another variable. Interactions were evaluated in the Poisson regression analyses.

Chi-square test: The Chi-2 test is used when data follows a nominal scale or ordinal scale and tests the differences in binary outcomes between two or more independent groups.

Fisher exact test: Like the Chi-2 test, Fisher exact test is used to test the differences in the proportion of positive outcomes between independent groups.

3.4.1 STUDY I

In study I, data was compared between the two cohorts using the Chi-squared test and Fisher exact test. The survival was estimated using relative survival calculated with the Ederer II method. Poisson regression analysis was used to

analyze the discrete variable number of deaths and to estimate excess mortality rate (EMR). The EEC regression model were adjusted for age, FIGO stage and tumor grade. The NEC regression model were adjusted for age, FIGO stage and histology. Interaction analyses were performed for cohort and stage, cohort and grade and cohort and age in the EEC group. In the NEC group, interaction analyses were performed for cohort and age and cohort and stage. The binary outcome treatment mode in cohort 1 and 2, was analyzed with Chi-square test and Fischer exact test to compare differences between the two cohorts.

3.4.2 STUDY II

In study II, both OS and RS were calculated. The Ederer II method was used to calculate RS. The reference mortality rate for each patient was calculated from the mortality rate tables for the Swedish population, stratified by calendar year and age. Poisson regression analysis was performed for the outcome "number of deaths" a discrete variable and to estimate excess mortality rate ratios for P53, SPF and DNA ploidy separate. The multivariate regression analyses were adjusted for age, FIGO stage and grade. Treatment was not included in the multivariate models since it was considered to be an intermediate variable that affect, hence adjuvant treatment should not be controlled for. Interaction analyses were performed for each exposure variable and stage, grad and age.

3.4.3 STUDY III

In study III, Chi-squared test was used to compare the proportions of stage between different educational level groups. Absolute incidence rates (IR) were estimated by model-based marginal means. Poisson regression analyses were used to estimate total and stage specific IRR of EEC and NEC separately. Interaction analyses were performed for education and age and education and year.

3.5   ETHICAL CONSIDERATIONS

In 1964 the World Medical Association adopted the Helsinki declaration which explicitly concerns doctors and is divided into three parts; basic principles, clinical research (subjects with the disease of interest is included) and non-clinical research (subjects without disease) (106). The basal principles contain requirement for informed consent and that all human research should be reviewed by an independent committee. It also concludes that research that is not performed in accordance with the Helsinki declaration should not be published.

Regional ethical review boards, containing both researchers and laymen, were set up in 2004 when the law of ethical review of research related to humans were implemented (SFS 2003:460). The regional ethical review boards were replaced by the Swedish Ethical Review Authority in 1st January 2019. All studies were approved by the Regional Ethical Review Board in Gothenburg.

In study I and II the cohorts were retrieved from the WSHCR Clinical Registry and from SQRGC at RCC West. All analyses were performed by statistician at RCC West, data is stored in an encrypted file, to which only statisticians at RCC West have access. All results were reported at a statistical group level. In study II important data were missing, and we applied for a supplement from the Regional Ethical Review Board concerning review of medical records and pathologic reviews.

Ethical approval for study I and II: The Regional Ethical Review Board in Gothenburg, ref 371-12, date of approval 12th of September 2012. Supplement T890-15, date of approval 23rd of November 2015.

In study III the cohort were retrieved from the WSHCR Clinical Registry and from SQRGC at RCC West. Data on socioeconomic status and immigration status were retrieved from Statistic Sweden. An encrypted data file with the cohort were sent to Statistics Sweden where linkage to the Statistics Sweden Population Registry and the Swedish Registry of Education was performed.

Anonymized data with unidentifiable sequence numbers was then delivered to the statistician at RCC West. The key to personal identification numbers is held at Statistics Sweden and will be destroyed after 10 years. All results were reported at a statistical group level.

Ethical approval for study III: The Regional Ethical Review Board in Gothenburg: ref: T557-15, date of approval 7th of July 2015, T991-17, date of approval 21th of December 2017.

4 RESULTS AND DISCUSSION

4.1 METHODOLOGICAL CONSIDERATIONS

4.1.1 STUDY DESIGN

Study I and II were retrospective observational cohort studies. Data was prospectively collected, before events of interests occurred, in the WSHCR Clinical Registry and SQRGC by clinicians. Study III, also a retrospective observational cohort study, based on the prospectively collected data from the WSHCR Clinical Registry, SQRGC and Statistics Sweden Population Registry and The Swedish Registry of Education. Observational studies have limited level of evidence compared to randomized studies but all cohorts included in this thesis were large and all studies controlled for known confounders. A weakness in study I was the use of a historic cohort, cohort 1. A secondary aim in study 1 was to examine treatment differences between cohort 1 and 2. We could not draw any conclusions of changed proportions of treatment and its association with survival. Association between different treatment and survival is optimally evaluated in randomized controlled trials like PORTEC-1, -2, -3 and GOG-99, -122 (51, 52, 92, 107, 108). The large cohorts size also admitted stratified analyses of associations between exposures and outcomes such as age in study I and stage I, grade 1-2 and ploidy in study II and for age in study III. There were no possibilities to compare risk-groups in study I or include comorbidity in study I and II since these data were not available. In study I there were three risk groups in the clinical protocol that corresponded to cohort 1 and five risk groups in the clinical protocol corresponding to cohort 2. Risk group were not registered in cohort 2.

4.1.2   SYSTEMATIC ERROR

Systematic errors, bias, is in opposite to random errors, not affected by study size (109). Systematic errors are broadly categorized into three main groups; confounding, selection bias and information bias. The internal validity is an expression of the reliability of the study results (109). Limited systematic errors and small random errors increases the internal validity. Observational studies always suffer from systematic errors to some extent. Due to systematic errors conclusions of causality could not be drawn in observational studies.

4.1.3   CONFOUNDING

Confounding is defined as confusion of effects (109). It occurs when the effect of the exposure is mixed with effects of other variables. Confounding is prevented by matching, restriction or randomization. Randomization benefits from preventing unknown confounders to be imbalanced, which restriction could not. Confounding could also be controlled for in the data analysis by stratification or by regression models. The association between exposure and outcome could be over- or underestimated by confounding.

Study I, II and III were restricted to histopathology, a categorical variable, since we defined it as a confounder. Histopathology was dichotomized, and the EEC and NEC groups were analyzed separately, and stage-specific incidences in study III. In study II, only the EEC group was of interest. We identified stage and grade, categorical variables, as confounders and therefor restricted the subgroup analysis to stage I, grade 1-2, EEC when comparing RS of aneuploidy and diploidy. In study I and II, the outcome measure was RS. RS could suffer from non-comparability of the external population if causes that influence mortality is distributed differently between the cancer group and the external cancer group. Increased BMI, lack of physical activity and unhealthy diet might be more prevalent in the cancer group compared to the population group when studying EC.

In study I histopathology was dichotomized into EEC and NEC. In table I, there is no evident imbalance of stage, grade or age in neither EEC nor NEC groups between cohort 1 and 2. Age was dichotomized into age <70 and ≥70 years old and stratified analyses of RS were performed in study I. Analyses of cohort 1 EEC found a significant different relative risk ratio for mortality in multivariate analyses between <70 and ≥70 years old which motivated the stratified analyses of relative survival by age. Univariate and multivariate Poisson regression analyses were performed for both EEC and NEC groups. In the EEC group the covariates were cohort, age, FIGO stage and grade. In the NEC group covariates were cohort, age, FIGO stage and histopathology. Comorbidity, BMI, LVSI and ethnicity were not included as confounders in the regression analyses since data was not available for these, which could result in under adjusted models (35, 57, 110) and residual confounding could be present due to confounders not included in the analyses or to misclassification of covariates .

In study II, only the EEC group were of interest. FIGO stage and grade, identified as confounders in study I were associated with SPF, DNA ploidy and p53. Age, dichotomized into <70 and ≥70 years. The Poisson regression analyses included covariates FIGO stage, grade and age together with DNA ploidy, SPF or p53. Residual confounding could be present due to misclassification of covariates and confounders not included in the analyses. The majority of included women did not have a lymphadenectomy, which means that patients in stage III could be misclassified as stage I and II. Misclassification of stage could bias the estimates in the multivariate analyses in both directions.

A study by Santala et al restricted the study to include only EEC and found DNA ploidy to be an independent prognostic factor in stage I (75). Unlike our study grade 3 was included and a Cox regression analyses demonstrated a significant association between DNA ploidy and relative risk of death, an

analyze we could not perform due to technical statistical reasons. As in study I, data was not available for known confounders and misclassification of covariates like grade and stage could result in residual confounding. Adjuvant treatment was considered an intermediate factor, therefore not adjusted for. In the study by Green et al, adjuvant treatment was not associated with 5-year endometrial cancer death in multivariate regression analyses (65).

In study III, the study population was restricted to EEC and NEC analyzed separate for stage-specific IR. Age and year of diagnosis were investigated for confounding with evidence of increased IR with increasing age in both stage I-IV EEC and the NEC group. There was no evidence of influence of year of diagnosis on IR in EEC (p=0.12) although this was evident in the NEC groups, p<0.001. Still there was no systematic time trend in incidence rate of NEC. Residual confounding could be present due to confounders not included in the analyses. Age at diagnosis was stratified into three age categories, 30-49, 50-74 and ≥75 year and there was a significant association between lower educational level and higher proportion of stage II-IV in the age group 50-74-year-old at diagnosis. Further stratification of age at diagnoses within the group 50-74 revealed that the proportion of stage II-IV EEC across the educational level differed less markedly in the age group 50-59 compared with the age group 60-74 years. Univariate analyses of the age group 50-74 years revealed a significant difference in IR in II and III-IV according to educational level. Poisson regression analyses were performed in the age group 50-74 year at diagnosis, to estimate total and stage-specific incidence rate ratios of EEC and NEC including covariates year of diagnosis, age at diagnosis and educational level or immigrant status.

4.1.4   SELECTION BIAS

Selection bias occur when selection of subjects is not representative for the target population, resulting in an association between exposure and outcome that differ from the non-participants (109). Study I, II and III included patients

registered in the WSHCR Clinical Registry and SQRGC. These clinical registers were matched against the obligatory Swedish Cancer Registry with a 99% coverage of the clinical registers in study I. Study II had a 99.5% coverage of registered EC between 2006-2011. Study III included women diagnosed with EC between 1995-2016, matching against the Swedish Cancer Register revealed a 95% coverage in the WSHCR Clinical Cancer Registry and a reported coverage of SQRGC at 95% (111) . Although the Swedish Cancer Registry have an estimated underreporting rate of 3.4% for female genital organs, the coverage of diagnosed patients is considered high (98). The high coverage data used qualifies study I, II and III as population-based. The WSHCR harbor 20% of the Swedish population, including the second largest city in Sweden. Randomized studies (51, 52, 92, 107, 108), with a selected study base are valid in the studied group and it might not be possible to generalize to a wider group, compared to study I-III which included patients in a geographical area, with high coverage. RS is not biased by selection which cancer-specific survival/mortality is where competing risks, death from non-cancer causes, precludes death from cancer (51, 65, 79, 105, 112).

Patients were only loss to follow up if patients moved out from the WSHCR, when estimating survival outcome in study I and II. In study I, the outcome of 55 women is not known. This represents 1.2% of the study population. The most prominent missing data were stage, 8% in the EEC group and 11-12% in the NEC group. This could have biased the results if those missing were mainly exposed or unexposed. In study III, 390 or 7% of identified patients were excluded due to missing data on histopathology, stage and educational level. Missing data were limited, but missing data on educational level were most prominent in the age group $\geq$ 75. Clinical stage was used when surgical stage was missing (7%). Clinical stage was standard before surgical stage was introduced, but clinical stage will classify some patients into lower stage. Missing data might cause an unpredictable bias. A study by Green et al demonstrated an association between SPF status and 5-year endometrial cancer

death, but missing data for SPF was prominent (65). Excluded subjects could cause selection bias if the association between exposure and outcome were different from the included women. The association is underestimated if exposed subjects with the outcome is excluded and overestimated if unexposed subjects with outcome is excluded. Selection bias was limited since all studies included in this thesis had high coverage and numbers of patient loss to follow-up were considered low.

4.1.5   INFORMATION BIAS

Erroneous collected data on exposure and outcome causes information bias (109). Data measured on a categorical scale are often referred to misclassification, which implies that the study subject is classified to an inaccurate group of exposure or outcome. Misclassification of subjects could be differential or non-differential. Differential misclassification exists when the error rate is different in the categories of exposure or outcome. Non-differential misclassification is not dependent on other variables and the error rate is the same in both categories of exposure or outcome groups. The association could be under- or overestimated when differential misclassification is present. The association tend to be underestimated with non-differential misclassification of dichotomous exposures.

Non-differential misclassification could be present in study I though cohort 1 and 2 corresponded to two different clinical guidelines. Cohort 2 defined as patients diagnosed between September 11th 2006 and forward and cohort 1 as patients diagnosed until September 10th 2006. It is reasonable to think that subjects in cohort 1 were treated according to guidelines introduced in cohort 2 and vice versa during a transitional period of time. This could have biased the estimates in both EEC and NEC towards 1, diluting the effects. All specimens underwent pathology review to confirm original pathology. This would reduce misclassification of histopathology and grade, since there could be marked discrepancies and intra-observer variability in histologic type and

grade (113). The outcome measure, survival and incidence rate in study I, II, and III, data is reliable due to personal identification numbers. Studies using cancer-specific survival or mortality could suffer from misclassification of the cause of death (105) due to low-quality data or when cancer-consequent death is classified as cancer-specific (65, 82, 114-117).

Analyses of SPF, DNA ploidy and p53 were performed on fresh frozen and/or paraffin-embedded hysterectomy and/or curettage specimens. It was not possible to discriminate between the proportion of hysterectomy versus curettage in the register. The concordance of DNA ploidy on fresh frozen and FFPE were reported 87% and SPF values from frozen sample were reported on average 1.5% lower than FFPE (118).Analyses of repeated sampling resulted in discordance in both SPF (17.8%) and DNA ploidy (11.8%). Patients were categorized as aneuploidy or SPF ≥8% if one of the specimens turned out so. The misclassification is considered to be non-differential since the error rate was the same in both the exposed and unexposed group. Misclassification of p53 was limited by independent evaluation by two observers after an initial joint session where scoring system based on intensity and extent of staining was agreed on. It is reasonable to think that there is undifferential misclassification of these dichotomous exposures which would dilute the estimates towards 1. Studies of DNA ploidy, SPF and p53 used different cut-offs compared to our study (65, 81, 112). If the cut-off is to low there is a possibility that the estimate will be diluted and an association will not be significant if the study base is too small. Correct measure of the exposure and outcome variables depends on the sensitivity and specificity of the diagnostic methods used. Image cytometry is a more sensitive method for DNA analyses compared to flow cytometry, the method used in study II. This could result in more misclassification in studies using flow cytometry compared to image cytometry (64, 119).

Level of education among immigrants are known for misclassification even when information on educational attainment is known (120). Level of

education would be a misclassification of a covariate, which were not used in the analyses due to this risk. If this covariate would be used in the multivariate analyses the confounding effect of educational level would not be fully controlled for, so called residual confounding. Data on the exposure variable education level and outcome variable incidence are reliable due to personal identification numbers and registry data. There could be undifferential misclassification EEC or NEC but we considerate it limited since pathologists reviewing all sample for histopathologic evaluation.

4.1.6   EXTERNAL VALIDITY

External validity implies generalization of the study results to individuals beyond the target population. External validity is dependent on high internal validity. Study I, II and III were all population-based studies with high coverage data. The WSHCR comprise 20% of the Swedish population distributed in rural areas and cities of varying size (121). National guidelines were introduced in 2013 in the WSHCR. Before this, regional clinical protocols were used. Both study I, II and III were performed on historic cohorts. RS takes into account an eventually increased survival over time in the population. The Swedish population could have changed in composition during this time. In 2000 12% of the population were foreign born females aged ≥45 years compared to 17% in 2016 in the (121). The WSHCR consist of the second largest city in Sweden, Gothenburg, both SES distribution and the proportion of foreign born could vary between cities and countryside. The external validity is low and the risk estimates in study I, II and III could not be generalized to the Swedish population or to other countries. Although the tree studies could provide information for similar settings in high income countries.

4.1.7   RANDOM ERROR

When systematic error is eliminated, random error, the variability in data due to chance, is left (109). Both systematic and random errors can distort estimated results in a study. Random error is reduced with increased study size. The confidence interval, express the underlying variation or statistical error and indicates the amount of random error in the point estimate. The level of confidence is the probability of including the true value in the confidence interval. The level of confidence was set to 95%, meaning that if the data collection and analysis were replicated 100 times the true value of the measure would be included in the confidence interval 95 times. This is only valid if there is no systematic error. A wide confidence interval means lower precision. The more observations and lower variability in data, the narrower confidence interval. In study I, II and III a 95% confidence interval was used.

The p-value is used for statistical testing of hypothesis. The p-value is a number between 0 and 1 that indicates how compatible the study results are compared with the null hypothesis. The lower p-value, the stronger evidence against the null hypothesis. In study I, II and III a p-value <0.05 was considered significant.

4.2   FINDINGS AND IMPLICATIONS

4.2.1   STUDY I

4.2.2   RELATIVE SURVIVAL IN COHORT 1 AND 2 EEC

The two cohorts were compared with respect to survival. The 5-year RS was estimated in the two cohorts. There were no differences in 5-year RS between cohort 1 and 2, 87.2% (95% CI 85.2-89.0) and 88.3% (95% CI 85.2-91.1). The survival rates were in accordance with previous studies of EEC stage I-IV

(122). Cohort 2 were not associated with decreased excess mortality rate in neither univariate nor multivariate Poisson regression analyzes. Changes in the clinical protocol used in cohort 2 were introduced to create a more individualized risk group classification with additional prognostic factors; SPF, p53 and age, since survival analyzes did not demonstrate any survival differences between stage I low and intermediate risk group in cohort 1(53). The changes mainly concerned the EEC group with lymphadenectomy performed in intermediate risk group B and C stage I with omitted EBRT, if there were no presence of lymph node involvement, and patients were treated with adjuvant VBT. This should lead to lower frequency of EBRT. We could not find any survival advantages of changes made in the clinical protocol. There is also, confirmed in randomized studies, no survival advantages detectable from lymph adenectomy (87, 88). The clinical protocol introduced in 2006 did not fall out in any increased survival in the EEC group.

The indication for adjuvant treatment were changed in the clinical protocol introduced in 2006. As mentioned before, negative lymph nodes in stage I intermediate group B and C resulted in omitted EBRT and adjuvant treatment with VBT. When treatment was analyzed, the combination of surgery and radiotherapy were increased from 30.9%, in cohort 1, to 38.6% (p<0.001) in cohort 2. The radiotherapy groups included both EBRT and VBT in the analyzes, consequently we did not discriminate if EBRT were reduced and VBT was increased in the intermediate risk group B and C as intended. The risk group classification in cohort 2 also resulted in patient considered low risk in cohort 1, were classified as intermediate risk A due to increased SPF, leading to adjuvant treatment with VBT. Both PORTEC-1 and GOG 99 randomized studies support restricted adjuvant radiotherapy to high-intermediate risk group, to reduce recurrence, with no evidence of any survival effect (51, 52). GOG 99 demonstrated a cumulative incidence of recurrence in the observation group at 26% compared to 6% in the radiotherapy group. PORTEC-1 demonstrated a 5-year locoregional recurrence rate at 4% in the radiotherapy

group and 14% in the control group. PORTEC-2 randomized trial demonstrated that high-intermediate risk could be treated with VBT instead of EBRT without any significant differences in vaginal recurrences (p=0.39), locoregional recurrences (p=0.42), distant metastases (p=0.79), DFS (p=0.89) or OS (p=0.66) (92). In accordance with these studies, we did not find any survival advantages of increased RT or of assumed VBT as the only adjuvant therapy in intermediate risk B or C. In cohort 1 cisplatin and epirubicin were used and changed to paclitaxel and carboplatin in cohort 2. The proportion of chemotherapy were not changed between the two cohorts, 15.6% vs 15.7%, and there was no evident survival benefit with changed chemotherapy treatment. PORTEC-3, used four cycles of carboplatin and paclitaxel after combined EBRT and cisplatin, and did not demonstrate any OS benefit in EEC grade 3 or stage I-III with chemotherapy compared to pelvic radiotherapy (107). GOG-249, a randomized study compared VBT+chemotherapy with pelvic ERBT, in stage I-II high-intermediate and high-risk groups, including stage I EEC, reported a betters locoregional control in the ERBT arm and more adverse events in the chemotherapy arm, and concluded that EBRT should remain standard care of high-risk early-stage EC patients (123). Our study was designed to estimate RS in the two cohorts and EMR in cohort 2 compared to cohort 1, recurrence and recurrence-free survival were not included endpoints. We conclude that changes in the clinical protocol in cohort 2 was not associated with decreased excess mortality rate compared to cohort 1.

Prognostic factors like age, grade, histopathological type and LVSI are aggregated into different risk stratification systems to guide surgery and adjuvant treatment. The major risk stratification systems for early-stage EC used in the PORTEC-1, GOG 99, SEPAL, ESMO and ESMO-modified classification, were evaluated with respect to recurrence and nodal metastases(124). The ESMO-modified classification had the highest discrimination for recurrence-free survival and nodal metastases but none of these had high accuracy in stratifying risk of recurrence or nodal status. The

ESMO-ESGO-ESTRO consensus panel recommend risk group classification to guide adjuvant therapy, published in 2016, based on stage, histology, grade, MI and LVSI (78) divided into four groups; low, intermediate, high-intermediate, advanced and metastatic. We were not able to compare RS between the risk-groups in cohort 1 and 2 in stage I, since risk-group were not registered in the registries in cohort 2. The multivariate Poisson regression analyzes revealed an increased EMR in age $\geq$ 70 years, stage II-IV and tumor grade 2-3. The age cut-off was set to <70 and $\geq$70 years, since this cut-off was used in the clinical protocol in cohort 2 and Paulsson et al demonstrated age with cut-off at 70 as a prognostic factor for overall cause specific survival, HR 1.634 (95% CI 1.248-2.138) (53). The independent prognostic associations of FIGO stage and grade are in accordance with other studies (64, 117). Our results support the inclusion of these variables into a risk stratification system.

4.2.3 RELATIVE SURVIVAL IN COHORT 1 AND 2 NEC

The NEC group was analyzed separately and cohort 1 and 2 were compared according to survival. The 5-years RS was 45.6% (95% CI 39.7-51.4) in cohort 1 and 51.3% (95% CI 41.5-60.2) in cohort 2. The RS was essentially lower than the NEC group which are in accordance with previous studies (125). In Poisson regression analyzes, including the covariates histologic subtype, stage and age, the EMR in cohort 2 was significantly lower, EMRR 0.62 (95% CI 0.44-0.87, p=0.006) compared to cohort 1. We analyzed the distribution of treatment in cohort 1 and 2 and revealed significant differences with 10% fewer patients decline or not tolerate treatment and 16% more patients treated with adjuvant radio- and chemotherapy in cohort 2 compared to cohort 1. A Cochrane meta-analysis published in 2011, found a decreased risk of death of any cause, RR 0.88 (95% CI 0.79-0.99) when chemotherapy was compared to any other arm (89). When platinum-based chemotherapy regimens were compared to no chemotherapy, the relative risk of death were 0.85 (95% CI 0.76-0.96). In subgroup analyzes of NEC (clear cell and serous papillary

carcinoma) there were no over-all or progression-free survival advantages with chemotherapy, HR 0.98 (95% CI 0.68-1.40) and HR 0.84 (95% CI 0.57-1.23) but the analyzes had small numbers and therefore a lack of power. Subgroup analyzes of serous and clear cell carcinoma in the NSGO-EC-9501/EORTC-55991 randomized study did not find any significant differences between radio-chemotherapy vs radiotherapy in PFS, OS or cancer-specific survival(90). The PORTEC-3 study, including stage I-III serous and clear cell histology, randomly assigned patients to radiotherapy or radiotherapy and chemotherapy consisting of two cycles cisplatin during RT followed by four cycles of carboplatin and paclitaxel(107). Subgroup analyzes of serous cancer demonstrated no significant differences in failure-free survival, HR 0.63 (95% CI 0.36-1.12) between the arms but this analyzes also lack power. The ongoing GOG-258 randomized phase III study evaluates if carboplatin and paclitaxel are more effective with or without cisplatin and radiotherapy (126). This study included serous and clear cell carcinoma, which constitute about 20% of the study base. OS data is still premature for comparison. The value of chemotherapy in the NEC group is still unclear yet the reduced 5-years RS of NEC calls for a more effective treatment. The excess mortality rate in cohort 2 were 38% lower compared to cohort 1. This could be due to fewer patients declining or not tolerating treatment and it might be due to increased adjuvant chemotherapy. Therefore, there is a need for randomized controlled trials of adjuvant chemotherapy in the NEC group without a lack of power.

4.2.4 STUDY II

4.2.5   PROGNOSTIC EFFECT OF P53 AND SURVIVAL IN EEC STAGE I-III

We analyzed the prognostic effect of overexpression of p53 in EEC stage I-III and in stage I, G1-2. The 5-year RS in the p53 overexpression group was significantly lower compared to the p53 negative group, 98% (95% CI 94-100)

vs 84% (95% CI 77-90%). Despite this, we could not confirm p53 overexpression to be an independent prognostic factor for survival, EMRR 1.53 (95% CI 0.79-2.97, p=0.208) when adjusted for stage, age and grade. In stage I, G1-2 EEC, the 5-year RS was 10.95 (95% CI 0.87-1.07) in the p53 negative group and 1.02 (95% CI 0.98-1.04) in the p53 positive group. The integrated genomic characterization of endometrial carcinoma, published 2013, demonstrated a genome-based classification of EC. Four molecular categories were identified with array- and sequencing techniques, analyzing the integrated genomic structure instead of single mutations. Group 1) POLE mutation -ultra mutated polymerase ε mutated POLE, a subunit of DNA polymerase involved in DNA replication and repair, with high mutation rate and hot-spot mutations in POLE, 2) mismatch repair deficiency - microsatellite instability hypermutated, microsatellite instability mainly because of MLH1 promotor methylation, 3) copy number low, without a specific driver mutation-microsatellite stable and high frequencies of CTNNB mutations, 4) TP mutations-copy number high, TP53 mutations(17). The POLE ultra-mutated group contained mostly high-grade EEC. The microsatellite hypermutated group has same features as Lynch syndrome (7). Type I EC corresponded with the copy number low-group and type II EC with the copy number high-group. The copy-number high (serous like) group included 25% of EEC G3 (17), with a similar phenotype to uterine serous carcinoma including TP53 mutations with increased protein expression. Analyses of PFS found the copy-number high group to have the poorest PFS of the four groups. We could not confirm p53 overexpression to be an independent prognostic factor, which is not in accordance with several other studies (81, 82, 116).

Talhouk et al compared a molecular classification system, ProMisE, based on The Cancer Atlas genomic subgroups with ESMO risk-stratification system. found p53, detected by IHC, to be an independent prognostic factor for OS and DSS, HR 2.61 (95% CI 1.27-5.72) and HR 2.28 (95% CI 1.02-5.58). The cohort differed from ours in many ways; it consisted of both NEC and EEC

(76.4%), it was more prognostic unfavorable with higher rates of advanced stages including stage IV and G3 and finally, of all p53 positive patients only 19.8% were EECs. Another study of IHC detected p53 in EEC stage I-IV demonstrated p53 in combination with ASRGL1 (an independent prognostic factor in EEC) to be the best predictor of disease-specific survival (116). When categorized into risk-groups the intermediate (aberrant p53 and ASRGL1>75% or p53 wild type and ASRGL1≤75%) and high (p53 aberrant, ASRGL1≤75%) risk-groups were associated with increased risk of dying from disease, HR 3.93 (1.46-10.57) and HR 20.38 (7.19-57.72) adjusted for stage. Stelloo et al analyzed the prognostic impact of p53 expression, detected by IHC, in EEC stage I (and stage IIA). The disease specific survival was significantly lower in the p53 positive group (p<0.001). In multivariable analysis p53 positive was a strong independent prognostic factor for both recurrence and over-all survival, HR 3.777 (95% CI 2.364-6.37), when adjusted for age, grade, myometrial invasion, LVSI and treatment, molecular subgroup and L1CAM. Differences in results compared to our analyses of the full cohort, stage I-III and of subgroup stage I EEC grade 1-2 could be explained by the different cut-offs used. In Stelloo et al specimens were considered positive when >50% of the tumor cells showed strong positive nuclear staining or when discrete geographical patterns showed >50% of tumor cell positivity. Huvila et al considered P53 aberrant if cancerous cells were completely negative or presence of moderate-strong staining in >75% of tumor cells. Our cut-off, set to >30% strong nuclear staining, could be too low to detect differences between p53 positive and p53 negative groups. Today, p53 is classified as aberrant when tumor cells are completely negative and mutated when presence of >80% of tumor cells show strong positive staining. The cut-off used in the study, was set to predict positive lymph nodes; an association we did not evaluate in our study. P53 were analyzed on pre-operative curettage or biopsy and hysterectomy specimens. We do not believe that this could skew the estimates since the concordance rate of p53 between biopsy and hysterectomy specimens

are considered high, 97.9 (127) and presence of intratumor heterogeneity limited, concordance of blocks was estimated to 93.9% (128). The prognostic effect of p53 overexpression was not demonstrated in this study possible due to a too low cut-off value.

4.2.6 PROGNOSTIC EFFECT OF SPF≥8% AND SURVIVAL IN EEC STAGE

In the s-phase, one of five phases in the cell cycle, the DNA-replication takes place, therefore the SPF is a marker of proliferative activity. We analyzed the SPF in both stage I-III EEC and in the subgroup stage I, G1-2. SPF was used in cohort 2 to discriminate between low-risk group and intermediate risk group A if SPF ≥ 8%. This would result in adjuvant therapy with VBT if there was no presence of P53 overexpression, deep myometrial invasion, NEC, aneuploidy or G3 in stage I EEC. We revealed a lower 5-year RS in the SPF ≥8% group compared to the SPF < 8% group, 0.89 (95% CI 0.83-0.93) vs 0.97 (95% CI 0.94-1.00). Still SPF ≥8% in multivariate Poisson regression it was not significantly associated with increased excess mortality rate, EMRR 1.31 (95% CI 0.68-2.53). Subgroup analyzes of stage I EEC G1-2, SPF≥8% were not associated with decreased 5-year RS, the group treated with VBT if SPF was increased. This result is in accordance with other studies (75) and supports omitting SPF in the risk assessment to tailor adjuvant treatment.

Studies of SPF as a prognostic factor in EEC is limited and varies in survival outcomes. This could be due to lack of an established cut-off value for SPF, different techniques for measuring DNA content, and finally SPF could be analyzed on fresh-frozen or paraffin-embedded tissues from both curettage and hysterectomy specimens. The cut-off value used in our study was calculated at the laboratory. Kaleli et al studied SPF in EEC stage I-IV, calculated a cut-off value at 6% to discriminate groups with statistically differences in survival (74). Green et al used the mean SPF value to construct two groups of high

respectively low SPF with cut-off 5.5% before any further analyses were performed (65).This study was like ours a retrospective population-based study of stage I EEC G1-3 and used flow cytometry for analyze of SPF. They demonstrated an independent effect of SPF on endometrial cancer death, HR 2.6 (95% CI 1.5-4.5) adjusted for age, FIGO stage, grade, DNA ploidy and adjuvant treatment, but 21% of patients could not be analyzed for SPF because of background debris. This could have skewed the results. SPF were analyzed on both fresh frozen and paraffin-embedded tissue. The majority of samples were paraffin-embedded and the values of SPF are 1.5% lower (mean difference) in fresh frozen tissue compared to paraffin-embedded specimens according to Gudmundsson et al (118). Overall, this makes it difficult to completely compare studies of SPF even if we find no evidence that SPF is an independent prognostic factor for survival in EEC.

4.2.7   PROGNOSTIC EFFECT OF ANEUPLOIDY AND SURVIVAL IN EEC STAGE I-III AND STAGE I GRADE 1-2

We studied DNA ploidy and its prognostic value sin EEC stage I-III and in stage I, G1-2, because the WSHCR clinical protocol used between 2006-2011 included DNA ploidy in risk group assessment. We found aneuploidy to be associated with stage and grade, with increasing proportions of aneuploidy in higher stages and grades in accordance with previous studies (65, 115). In stage I-III the 5-year RS survival in patients with aneuploidy were significantly lower than in patients with diploid tumors, 0.82 (95% CI 0.75-0.87) vs 0.98 (95% CI 0.95-1.01) but DNA ploidy were not significantly associated with increased excess mortality, EMRR 1.70 (95% CI 0.89-3.24, p=0.107) when adjusting for stage, age and grade. If aneuploidy were present in patients with stage I EEC, G1-2, myometrial invasion <50%, would be classified as intermediate risk group. Aneuploidy together with G3 or myometrial invasion >50%, patients would be considered high risk. Therefore, we evaluated the prognostic effect of ploidy status in the subgroup FIGO stage I, G1-2, EEC.

Aneuploidy revealed a significantly lower 5-year RS (0.93, 95% CI 0.78-0.96) compared to DI, 1.02 (95% CI 0.99-1.05). It was not statistically possible to analyze the effects on excess mortality since there was no overall excess risk in this group, but we did find a significant association between DNA ploidy and grade (p<0.001). Therefore, we stratified for grade and demonstrated that aneuploidy grade 2 had the lowest 5-year RS, 0.88 (95% CI 0.78-0.96) and that diploidy grade 2 had no effect on 5-year RS. Aneuploidy might identify patients in the low risk group with impaired survival but this must be confirmed in further studies. The current clinical protocol for EC in Sweden recommends continuous collecting of DNA ploidy but it does not tailor the surgical procedure or adjuvant treatment.

Comparing the results from different studies is problematic, since the study populations, groupings, specimens analyzed, methods, and cut-offs often differ between diploidy and aneuploidy studies. We included tetra-ploidy in the aneuploid group and considered DNA index 0.96–1.04 as diploid. Studies of stage I EEC are limited and conflicting (65, 75, 119) due to these circumstances. Santala et al analyzed ploidy on fresh sample and demonstrated ploidy to be an independent prognostic factor for OS. Steinbakk et al analyzed paraffin-embedded curettage specimens with image cytometry and did not find ploidy status to be associated with death in EC. A large amount of missing data due to technical reasons and no events in the aneuploidy group makes the result unreliable. A large retrospective study (n=1069), using flow cytometry, did not demonstrate an independent prognostic effect of ploidy status for either EC related death, OS or time to progression. Mauland et al found a significantly lower disease-specific survival for aneuploidy in both EC stage I-IV and EEC stage I-IV, compared to diploidy (p=0.001, p=0.003) and also, aneuploidy to be an independent prognostic factor for survival when adjusted tor age, stage, histologic subtype and grade, (115). Analyses were performed on fresh samples mainly from hysterectomy specimens. Our analyzes were performed on both curettage and hysterectomy specimens but we could not discriminate

the proportion of the two. We found a discordance of DNA ploidy in 11.8% and of SPF in 17.8%. DNA heterogeneity between curettage and hysterectomy is described as higher when analyzed with image cytometry, 27,3% (129), and associated with tumor heterogeneity. Specimens with tumor heterogeneity had higher range of DNA index (1.14-2.02) compared to specimens with tumor homogeneity, which were all EEC G1-2, with variation in DNA index (1-1.2) We did not analyze the range of DNA index in the discordance of DNA ploidy but it may be limited since the majority of the cohort had EEC grade 1-2 (83.6%). DNA ploidy could be analyzed with flow cytometry or image cytometry. Image cytometry is a more precise method, and flow cytometry could misclassify small subpopulations of aneuploid and polyploid tumor cells as diploid (130). A study of EEC stage I-II (n=937), measured ploidy status with image cytometry in paraffin-embedded specimens from both curettage and hysterectomy, did not demonstrate an independent effect on overall survival in multivariable analyses (64). DNA ploidy reflects copy-number alterations and DNA ploidy was evaluated in the ProMisE molecular subgroups in EC (114). Abnormal DNA content was associated with a significantly worse overall survival but the study was underpowered to detect prognostic changes of abnormal DNA content in the subgroups, who all contained a proportion of abnormal DNA content. Further studies of DNA ploidy should be evaluated in a large cohort with optimal methodological management and preferably in relation to the ProMisE subgroups.

4.2.8   OTHER PROGNOSTIC FACTORS

There are several other prognostic factors of interest. Circulating biomarkers like plasma growth differentiation factor-15 (GDF-15), CA-125 and human epididymis protein 4 (HE4) needs further evaluation of their prognostic effect (131, 132). L1CAM, promotes cell motility and thereby invasiveness. L1CAM overexpression, detected by IHC, is associated with NEC, G3 and high-risk group in stage I (133) and further, in multivariate analysis, L1CAM, is an

independent prognostic factor for OS, HR 2.05 (95% CI 1.41-2.98). When L1CAM was included in multivariate analyze with ProMisE subgroups, it was not found to be an independent prognostic factor for OS, HR 1.33 (95% CI 0.77-2.22), or DSS, HR 2.05 (95% CI 1.00-4.10, p=0.05) (134). In the subgroup p53 wild-type/no specific molecular profile, L1CAM was an independent prognostic factor for DSS, HR 3.80 (95% CI 1.10-12.16) on preoperative biopsies and remained independent in analyses on hysterectomy specimens, HR 4.03 (95% CI 1.11-13.74). Stathmin, an oncoprotein that destabilize microtubule and allow cells to transit through the mitosis faster, is found to be an independent prognostic factor for DSS, HR 1.68 (1.05-2.67) (135). Presence of hormone receptors, ER and progesterone receptor, in EC is also demonstrated to be independent prognostic factors with increased DFS compared to receptor-negative tumors (136). When stathmin, progesterone receptor, ER and PTEN were evaluated in the context of ProMiseE subtypes, none of these factors added prognostic information over ProMisE subgroups (137). Evaluation of prognostic factors should be performed together with and within subgroups defined by the Cancer Genome Atlas Research project, to reveal patients with impaired prognosis.

4.2.9 STUDY III

4.2.10 EFFECT OF EDUCATIONAL LEVEL AND INCIDENCE OF STAGE-SPECIFIC ENDOMETRIAL CANCER

We studied the association between SES and IR of stage specific EEC and NEC. The IR of stage specific EEC increased from the age of 45-49 and continued to increase to the age of 80-84 in stage II-IV. The higher age at diagnosis in higher stages reflects a delayed diagnose. In EEC the age group 50-74 years, we found a significant association between educational level and stage distribution with higher proportions of stage II-IV in patients with low

educational level. No such association was evident in the NEC group, but the low number of events indicate lack of power. Further analyses of the age group 50-74 years revealed a significant association between educational level and incidence rates of stage II and III-IV and multivariate analyzes demonstrated an independent association between low educational level and incidence rate of stage II and III-IV compared to high educational level, adjusted for age at diagnosis and year of diagnosis.

We studied the association between educational level and the incidence of EEC. There are several indicators for socioeconomic status like educational level, income and occupational social class. These indicators reflect different aspects of socioeconomic status and therefore different health impact (36). Education may provide knowledge and competencies to avoid unhealthy behavior and smoking, alcohol, lack of physical activity, inadequate diet and high BMI are more prevalent among women with low educational level and income (36, 138). Both overweight, obesity (139) and the metabolic syndrome (140), a cluster of conditions with abdominal adiposity, dysglycemia, high blood pressure, elevated triglycerides and low HDL-cholesterol, is associated with increased risk of EC, RR 1.32 (95% CI), RR 2.54 (95% CI 2.11-3.06) and RR 1.89 (95% CI 1.34-2.67). Type I, G1-2 EEC, is estrogen dependent and the main source of estrogen in postmenopausal women is adipose tissue and the proportion of EEC histology is higher in patients with increased BMI as opposed to NEC (110). We found an association between level of education and total incidence rate although it was modest and only present in the low and not in the intermediate level of education, IRR 1.11 (1.01-1.22) and 0.98 (0.98-1.18) compared to high level of education in the age group 50-74-year-old.

Stage distribution according to education level revealed higher proportions of stage II-IV in patients with low educational level in EEC. This was only present in the age group 50-74-year-old and was not found in the NEC group. We demonstrated significant associations between level of education and IR of stage II and III-IV and women with low, compared to high, educational level

had higher incidence rates of stage II and stage III-IV EEC, IRR 1.65 (1.13-2.42) and 1.82 (1.33-2.49) in the age group 50-74-year-old. EC in childbearing age is rare, only 4% are <40-year-old, and tend to have more early-stage EC (141). The association between lower educational level and increased incidence rate of stage II and III-IV EEC in women aged 50-74 years could be due to patient and/or doctors delay. Doctors delay could be due to variations in positive predictive value among patients with EEC according to age where vaginal bleeding is an alarm symptom in postmenopausal women, leading to a higher proportion of fast-track referrals, compared to irregular bleedings in younger and perimenopausal women (142). When the age group 50-74 was divided into two, age 50-59 and 60-74, the proportions of stage II-IV differed the most in the age group 60-74 years. More frequent delays in contacting general practitioner or gynecologist when irregular or postmenopausal vaginal bleeding occur, among those women with low educational level, could be due to gaps in health literacy including false sense of security when participating in cervical screening, difficulties in communications with health services and absence of feeling unwell together with sudden presence of vaginally bleeding, (143).

4.2.11   EFFECT OF IMMIGRANT STATUS AND INCIDENCE OF STAGE-SPECIFIC EC

Despite a higher proportion of low level of education among foreign-born women compared to Swedish-born women (46.6% vs 38.1%) with EEC aged 50-74 years there were no association between immigrant status and higher IR of neither stage I EEC nor stage II-IV EEC, IRR 0.91 (0.80-1.04), IRR 0.91 (95% CI 0.69-1.20). The group of foreign-born, 11.2% of the identified patients, was small (n=652) and heterogenous; it consisted of both women with Scandinavian ethnicity in 46% (n=301) and non-Scandinavian ethnicity. Our results could be explained by lack of power and data on level of educational level is often misclassified among immigrants the first five years since arrival

in Sweden, due to high prevalence of missing data and educational misclassification (120). A Swedish study from 2009 found an over-all lower adjusted RR of EC in foreign-born women, RR 0.79 (95% CI 0.75-0.83). The risk varied by country of birth and women from eastern Europe, Poland, Bosnia and Africa did not differ significantly from Swedish-born women (144). Our study included female immigrants between 2000-2016. Immigrants have been described to have a better health than natives, the so called "healthy immigrant effect" explained by self-selection of the most motivated and healthies to migrate, migration policies select more educated/wealthy/healthier immigrants, healthier lifestyle in the home country concerning diet, physical activities, socially protective factors and a potential under-reported health upon arrival (145). This health advantage declines with time towards the health status of the local population (145). For breast cancer the adjusted RR of stage II was 1.09 (95% CI 1.00-1.19) among foreign-born compared to Swedish-born women (146). The effect is uncertain but delay in diagnose could be due to low adherence to mammographic screening, language barriers, cultural behavior, unclear access to medical service.

4.2.12   AWARENESS AND SCREENING PROGRAMS

Women should be informed about risk factors, preventing factors and symptoms of EC. This includes encouragement of physical activity and a healthy life style to maintain a normal weight and blood pressure, and avoiding diabetes, which all are risk factors for EC. Women in menopause age should be informed about symptoms of any vaginal bleeding or spotting and strongly encourage to seek a doctor to prevent increased stage at diagnosis. This is even more important in women with risk factors like obesity, tamoxifen therapy, late menopause, nulliparity. Postmenopausal women with tamoxifen is recommended monitoring of symptoms but routine endometrial surveillance is not proven effective in increasing early detection of EC (147).

Screening is not recommended in asymptomatic women with average risk of EC, since there is no appropriate screening test available. According to National Cancer Institute there is no evidence that ultrasound decreases mortality in EC and will result in unnecessary biopsies (148). Biopsy as a screening method lack sufficient evidence of decreased mortality in EC. Lynch syndrome mutation carriers, women with mutation carriers in the family and women in families with autosomal-dominant predisposition to colon cancer should undergo annually biopsies from age 35 (149).

In Sweden screening program for colon-rectal cancer was implemented in 2008 in the Stockholm-Gotland region and will be implemented in the WSHCR in 2019. Both EEC and colorectal cancer in patients with lower SES are associated with increased incidence rates in stage II-IV in the same age groups (150). De Clerk et al examined organized colon-rectal cancer screening programs with data on SES; 90% reported lower participation rates in the lower SES groups (151). Participation rates varied from small 66% vs 71% to marked 35% vs 61%, in patients with low compared to high SES (151). We encourage an awareness program, coordinated with the expected implementation of a screening program in WSHCR, to reduce both patient and doctors delay. Implementation of such awareness program should optimally be performed as a randomized health service study (152). This design provides valid large-scale data in accordance with evidence based medicine and lower risk of bias compared to observational study design (152). Cluster-randomization could be an option in this case.

5 CONCLUSION

- We found the clinical protocol used in non-endometrioid endometrial carcinoma, cohort 2, to be associated with a significantly reduced excess mortality rate compared to cohort 1.

- We did not find the clinical protocol used in endometrioid endometrial carcinoma, cohort 2, to be associated with reduced excess mortality rate compared to cohort 1.

- We found age ≥70 years to be associated with increased excess mortality rate in both endometrioid and non-endometrioid endometrial carcinoma.

- We found significant differences in distribution of treatments between cohort 1 and 2 in both endometrioid and non-endometrioid endometrial carcinoma.

- In stage I grade 1-2 endometrioid endometrial carcinoma, aneuploidy identified patients with impaired 5-year relative survival.

- We did not find p53 overexpression, SPF $\geq8\%$ or aneuploidy to be associated with increased excess mortality in stage I-III endometrioid endometrial carcinoma when adjusted for stage, grade and age.

- Lower level of education is associated with higher incidence rates of stage II and III-IV endometrioid endometrial carcinoma compared to high level of education in 50-74-year-old women

- We did not find any association between level of education and stage in non-endometrioid endometrial carcinoma.

- We did not find immigrant status to be associated with increased incidence rates of stage II-IV endometrioid endometrial carcinoma in women aged 50-74-year-old.

6 FUTURE PERSPECTIVES

- Studies of adjuvant therapy should preferably be conducted as randomized controlled trials with recurrence and recurrence-free survival as primary or secondary endpoints.

- Randomized controlled trials of adjuvant chemotherapy in the NEC group is needed to optimize adjuvant treatment and hopefully increase survival.

- Prognostic factors should be evaluated together with and in the four distinctive cancer genome based prognostic subgroups.

- Reassessment of risk-group classification, taking into account both clinicopathological features and biomarkers in EC is needed to improve risk assessment of patients and to tailor adjuvant treatment.

- Further studies of stage specific incidence rate and SES including level of education and household income might identify age groups with increased incidence rate of increased II-IV stage of EEC.

- Awareness programs addressed to identified population groups with increased incidence rate of stage II-IV EEC should be evaluated in health service trials.

- Coordination of awareness programs for EC and screening for CRC could be studied in a targeted health service trial for improved EC awareness and colorectal cancer screening.

ACKNOWLEDGEMENT

I am deeply grateful to everyone who has contributed or in any other way made this thesis possible.

A special thanks to:

Professor *Karin Sundfeldt*, my principal supervisor, for inspiring enthusiasm, never-ending encouragement, kind guidance and patience with my shortcomings. Your excellent scientific skills, always focusing on the essential matters and keeping me on the right track have been crucial.

Janusz Marcickiewicz, MD, PhD, head of the regional process of EC at RCC West, my co-supervisor, for your generous contribution with your vast knowledge of EC to this project. For believing in me and having the courage to turn our collaboration into this project.

Professor *Ulf Strömberg*, my co-supervisor, for enthusiastic support and educational skills, sharing your extensive knowledge in epidemiology so generously with me throughout this project.

Erik Holmberg, PhD, for contributing with your outstanding knowledge, interesting discussions and guiding me into a deeper understanding of statistics.

Herman Nilsson-Ehle, MD, PhD*, Katja Stenström-Bohlin*, MD, PhD, and *Klaus Groth,* MD, PhD, for constructive discussion at my half-time seminar.

Anja Andersson, for your helpfulness and administrative support.

Claudia Mateoiu, Ulla Delle, Rita Pedersen and *Annika Pontén,* for your educational support.

Eva Hessman and *staff at the Biomedical Library,* for all help and support.

All my colleagues and friends at the Department of Obstetrics and Gynecology, for your support, encouragement and not least, work while I have been doing this thesis.

Lena Otterlind, Radha Korsorski and *Mårten Alkmark,* present and former bosses, for making this project possible.

My family, my husband *Robin* and our wonderful children, *Josef, Johannes* and *Jacob*, for all your love, support and patience with me and this project.

Lennart and *Maria,* my beloved parents, for your unlimited love and always supporting me and my ideas. My sister and brothers *Alexandra, Martin* and *Peter,* with families, and my husband's family, so blessed to have you in my life.

All my wonderful friends that enrich my life, looking forward to see more of you now.

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