ENDOMETRIAL CANCER;

Obesity-related carcinogenesis and diagnostic innovations

Hannah Donkers

Stellingen behorende bij het proefschrift

Endometrial cancer; obesity-related carcinogenesis and diagnostic innovations

1. Vrouwen gediagnosticeerd met non-endometrioïde endometriumcarcinoom hebben een slechtere overlevingskans wanneer zij een hoog visceraal vet percentage hebben – *dit proefschrift*

2. Verlies van spiermassa (sarcopenie) heeft geen invloed op overlevingscijfers bij vrouwen met hooggradig endometriumcarcinoom – *dit proefschrift*

3. Bij vrouwen met een hooggradig endometrioïde endometriumcarcinoom leidt de combinatie van sarcopenie en obesitas tot een verminderde overleving – *dit proefschrift*

4. Vrouwen uit een laag sociaal milieu met endometriumcarcinoom sterven op jongere leeftijd, doordat zij met een hoger stadium gediagnosticeerd worden, ouder zijn en vaker te kampen hebben met overgewicht of obesitas – *dit proefschrift*

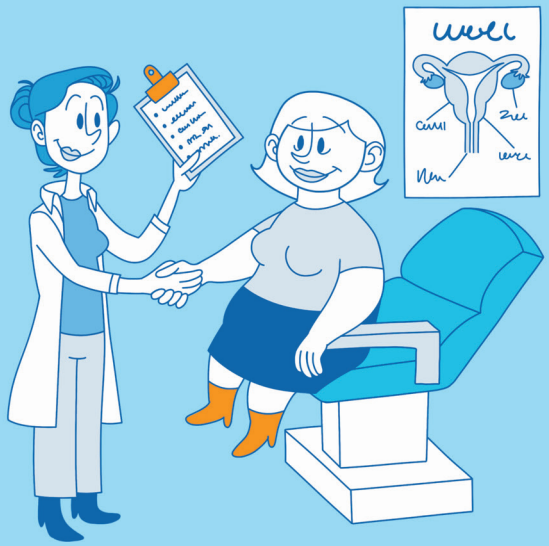
5. Anemie is een onafhankelijke prognostische factor in endometriumcarcinoom – *dit proefschrift*

6. De expressie van miRNA in urine of endometriumweefsel bij vrouwen met endometriumcarcinoom verschilt van de expressie van miRNA bij gezonde vrouwen – *dit proefschrift*

7. MiRNA expressie in urine is potentieel veelbelovend in de screening op endometriumcarcinoom bij vrouwen met abnormaal vaginaal bloedverlies – *dit proefschrift*

8. Everything before the ''but'' is meant to be ignored by the speaker; and everything after the ''but'' should be ignored by the listener
   – *Nassim Nicholas Taleb*

9. Niet kiezen is ook kiezen
   – *Stan Donkers*



Hannah Donkers,
2021

Endometrial cancer; obesity-related carcinogenesis and diagnostic innovations

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Drs. M. Beyene

Dit proefschrift draag ik op aan mijn vader

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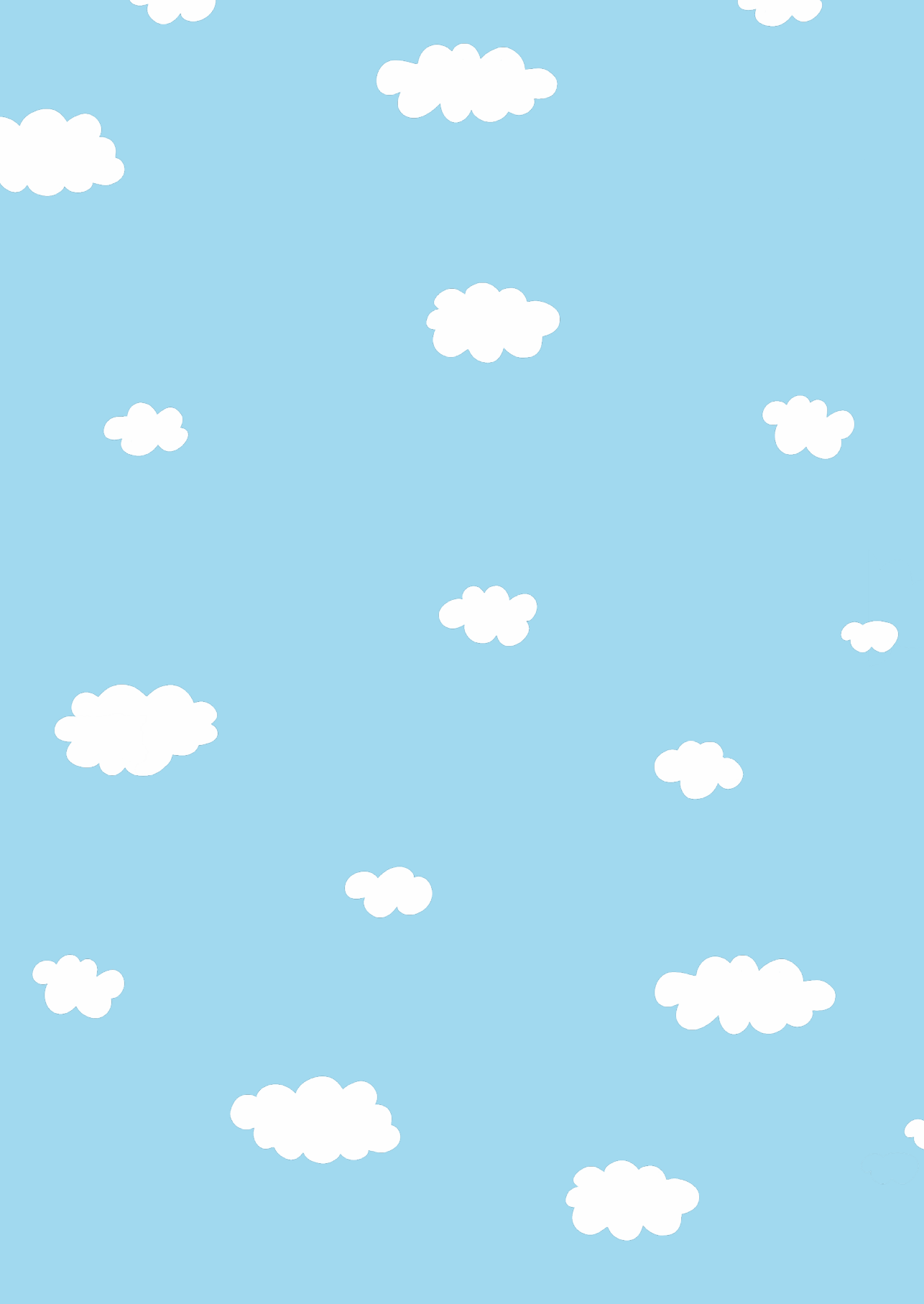

Chapter 1

Introduction, aim and outline of this thesis

Introduction

Epidemiology

Endometrial cancer (EC) is the sixth most commonly occurring cancer in women worldwide, with over 380,000 new cases and nearly 90,000 deaths in 2018[1]. In the Western world, it is the most common gynaecological cancer, affecting more than one in 20 female cancers in Europe[1,2]. Majority of ECs occur in postmenopausal women, with median age at diagnosis of 61 years, while up to 14% occur in women of reproductive age[3–8]. The primary symptoms of EC are postmenopausal bleeding (PMB) and abnormal premenopausal bleeding. As a patient's age increases after menopause, the probability that uterine bleeding is caused by EC progressively rises (**Figure 1**)[3,9,10].

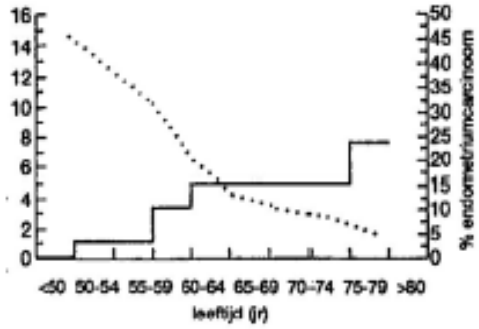

Figure 1. *Incidence of PMB per 1000 women (dotted line) and presence of EC in % (continuous line) in relationship to age*

As most patients present with PMB, they are diagnosed at early stage disease[11,12]. This results in an overall good prognosis with 5-years survival over 90%, when diagnosed with FIGO stage 1 EC[11,12]. However, survival rates vary for the different subtypes EC.

Tumour types

Generally, EC can be subdivided into two histological subtypes with differences in clinical outcomes[13–15]. Type I tumours that comprise the large majority of EC consist of endometrioid adenocarcinomas (EEC), are often preceded by endometrial hyperplasia and associated with unopposed oestrogen stimulation[16]. Type II tumours on the other hand consist of non-endometrioid tumours (NEEC) including serous carcinomas, clear cell carcinoma and carcinosarcoma. NEEC are commonly considered oestrogen independent, arising in atrophic endometrium deriving from intraepithelial carcinoma, as precursor lesions[16]. Type II tumours are poorly differentiated and have worse outcome than type I tumours[17]. The main risk factor

for the development of type I tumours is unopposed and prolonged oestrogen stimulation of the endometrium due to: obesity, metabolic syndrome, hormone therapy, nulliparity, early menarche and late menopause[18–21]. Although these risk factors have been mainly attributed to type I tumours, recent data illustrate that these are also related to type II tumours[16]. In addition, type II tumours are also associated with hyperinsulinemia and diabetes mellitus (DM) independently of Body Mass Index (BMI), further supporting the resemblances in pathophysiology and aetiology of EEC and NEEC[16].

Recent genomic characterisation of EC through The Cancer Genome Atlas (TCGA) Project classified EC into four integrated clusters: *POLE* ultramutated (POLE), microsatellite instability hypermutated (MSI), copy-number low (CNL) and copy-number high (CNH)[22]. The *POLE*, MSI and CNL clusters were composed mostly of endometrioid histology and most serous tumours were found in the CNH cluster[22]. *POLE* mutated tumours showed the best progression-free survival (PFS), while women with CNH tumours had the worst prognosis[22]. There appears to be an association between obesity and the EC integrated clusters: women with CNL tumours have the highest median BMI whereas women with *POLE* mutated tumours have the lowest BMI[23]. Furthermore, oestrogen receptors (ER) were found evenly expressed (50-75%) across all four subtypes, offering a potential pathway for sex hormones to mediate obesity-related cancer tumorigenesis in all four subtypes and therefore potentially explaining the link between increased BMI and risk for both type I and II EC[24].

EC carcinogenesis

During the development of EC, there is an imbalance between oestrogens and progestogens. Oestrogens are the primary female sex hormones responsible for endometrial proliferation[25]. Progestogens on the other hand counteract the oestrogen-induced endometrial growth, and induce differentiation into the secretory phase of the endometrium[26]. High levels of oestrogens unopposed by progesterone, as frequently observed in obese women, can therefore lead to endometrial hyperplasia, and subsequently develop into early-stage EC[27].

Carcinogenesis is a multistep process, starting from the initial carcinogenic stimulus, followed by changes at the cellular level with abnormal cell division, to the final manifestation of cancer. Although uncontrolled growth is a fundamental characteristic of cancer cells, these cells also require a proper microenvironment to survive, develop and progress[28]. The tumour microenvironment (TME) consists of extracellular matrix including myofibroblasts and cellular players, such as fibroblasts, neuroendocrine cells, blood and lymphatic vascular networks, immune-inflammatory cells and adipose cells[29]. Adipose cells play an important role in EC microenvironment because they establish a proinflammatory microenvironment,

which is more likely to breed tumours[30]. In addition, adipose cells secrete more than 50 different cytokines, chemokines, and hormone-like factors, which may be accomplices in tumour initiation (**Figure 2**)[31,32].

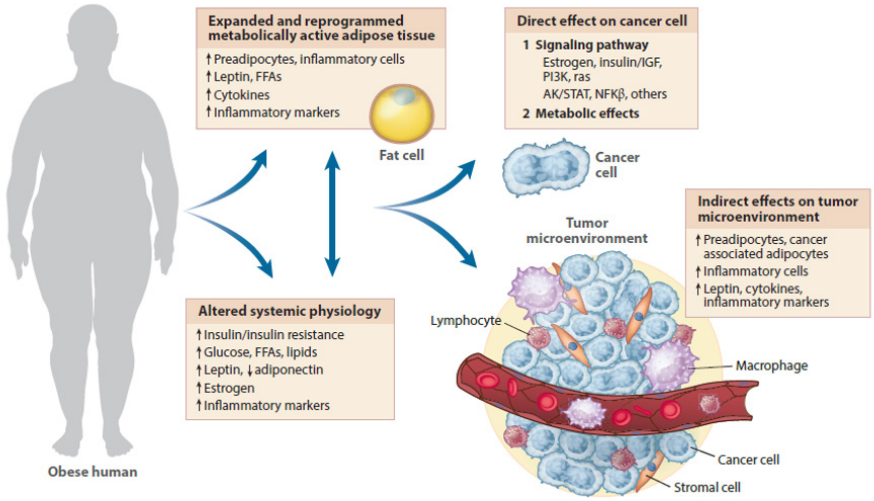

Figure 2. *Obesity is associated with metabolically active adipose tissue, leading to inflammation and altered cytokine/adipokine secretion. These changes contribute to and interact with alterations in systemic physiology that reflect the insulin resistance and metabolic syndrome. Adipose tissue can impact cancer directly by activating the signalling pathway and altering cellular metabolism but also indirectly on the tumour microenvironment to promote proliferation, angiogenesis and invasion.*

Whereas TME is seen as the local interaction between the cellular elements of a tumour, the tumour's macroenvironment can be described as the systemic interaction between the tumour microenvironment with other organs and systems in the body (**Figure 3**)[33].

Tumour-induced angiogenesis supports tumour growth, however the newly formed network of blood vessels are leaky, resulting in an accumulation and/or release of soluble factors from the TME into the circulation[33]. This leads to pathological endocrine effects and interaction between the TME and the patient's organs and systems, resulting in the development of cancer-associated systemic syndromes in the tumour macroenvironment[33].

The major influence of the tumour on the macroenvironment appears to be related to excess of cytokines in the serum, which are responsible for promoting degradative

pathways in skeletal muscle and adipose tissue[34]. The most well-known systemic, multi-factorial syndrome is cancer cachexia, which is characterised by progressive loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment[35]. Cancer cachexia links regulation of the macroenvironment of tumours to the entire individual.

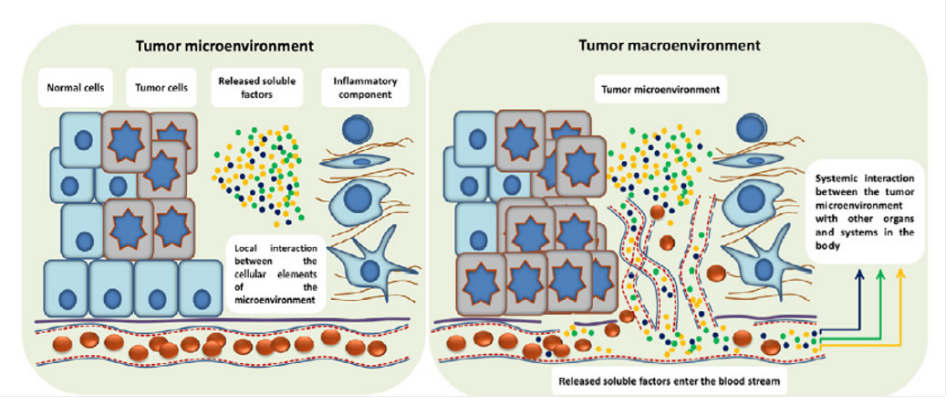

Figure 3. *The tumour micro- and macroenvironment concept.*

Obesity and EC

The rising incidence of EC has been attributed to the increased prevalence of obesity in developed countries for both type I and type II EC[36–40]. Obesity is also an important contributor for morbidity and mortality from several diseases such as cardiovascular disease, diabetes and different cancer types including EC[41]. Over the past 10 years, the World Health Organization (WHO) has recognised the increased number of people who are overweight or obese, and attention is now being given to the global implications associated with this trend[42]. Since obesity is a multifactorial problem, understanding and addressing obesity requires recognising and appreciating biology including mechanistic background, behaviour, environment and culture.

Individual, environmental, socioeconomic and genetic factors are all linked to the increase in obesity in developed countries[43]. The individual factors for obesity consist of diet, physical activity, sedentary behaviour and sleep[43]. Socioeconomic status (SES), which is based on income, education and occupation, is associated with obesity, in which obesity is more frequently seen in individuals with low SES[44,45]. Individuals of lower SES tend to be less physically active and have unhealthier food habits, contributing to poorer health status[46,47]. Low SES is associated with increased morbidity including DM and cardiovascular disease and

all-cause mortality including cancer-related mortality[48–50]. There seems evidence of a relationship between socioeconomic deprivation and survival in EC, however the exact link including the relationship with obesity is unclear.

The pathogenetic mechanisms for endometrial tumorigenesis in relation to obesity is explained by a variety of mechanisms and relevant in both type I and type II EC. In adipose tissue, both adipocytes and stromal cells express aromatase, an enzyme which converts circulating androgens (androstenedione and testosterone) to oestrone (E1) and oestradiol (E2), respectively[51–58]. The excess oestrogen produced by adipose tissue provides a growth signal for the endometrium that is unopposed by progesterone, leading to an increased risk of EC. Furthermore, like in most cancers, the TME also plays an essential role in EC progression[59]. Mutations in *PTEN*, *KRAS*, *p53*, and microsatellite instability initiate EC lesions, but this does not lead to high-grade cancer or metastasis unless supported by the microenvironment[59]. Furthermore, in obese women, the paracrine signalling from visceral adipocytes is associated with chronic inflammation mediated by proinflammatory adipokines leading to hyperinsulinemia, increases in insulin-like growth factor 1 (IGF1) and hyperglycaemia, which in turn also stimulates endometrial proliferation[61]. In addition, sex hormone-binding globulin (SHBG) levels decrease with increasing adiposity, thereby increasing the pool of bioactive oestrogen[60].

Obesity and body composition in EC

BMI is the most frequent used measurement to class individuals into underweight, normal weight, overweight and obese. However, BMI has limitations as a measure of obesity as it does not provide an appropriate assessment of body composition including differentiation between muscle and fat mass[62,63]. Therefore, attention has shifted towards the usage of body composition measurements. Body fat can be distributed into subcutaneous adipose tissue and visceral adipose tissue, which is defined as fat around the viscera and inside the intra-abdominal parenchymal organs. High percentages of visceral fat are associated with worse outcomes in several cancers sites such as breast and ovarian cancer[64,65]. In addition, visceral fat is associated with an increased cardiometabolic risk[66]. Furthermore, sarcopenia, described as a pathological decrease in muscle mass, is an objective measure of frailty and is associated with poor outcomes including survival for several cancer types[67,68]. The relationship between body composition and outcomes in EC patients, is unclear. Understanding these underlying mechanisms could aid in understanding the obesity-related EC carcinogenesis.

Obesity and haematological parameters in EC

The chronic low-grade inflammation state of obese patients is characterised by elevated levels of circulating pro-inflammatory cytokines and acute phase

proteins[69]. This inflammatory state generates a pro-tumorigenic environment promoting angiogenesis[70]. In addition, excessive cytokine production stimulates the release of young and large platelets from bone marrow to the peripheral blood, which cause changes in haematological parameters[71]. Due to their angiogenic, metastatic, and proteolytic activities, platelets have a major role in the background of inflammation and are an important part of the TME[72,73]. Pre-treatment thrombocytosis, often defined as platelet count higher than 400 x 10^9/L, has been associated with adverse prognosis in EC[74]. Furthermore, an inverse relationship between haemoglobin and thrombocyte levels is seen, and anaemia is thought to be a marker of tumour burden or biologically more aggressive disease[75]. In addition, preoperative leucocytosis, which is more often seen in obese patients, is associated with increasing tumour size and increased risk of death in EC[76,77]. It has been hypothesised that leucocytosis is also linked to the presence of comorbid conditions, however this association is unclear in EC.

Obesity and miRNA in EC

To fully understand the link between EC and its major risk factor, obesity, new insights into the pathogenetic and molecular mechanisms for obesity-related endometrial tumorigenesis are needed.

MicroRNAs (miRNAs) are a novel group of non-coding RNAs that have emerged as important regulators of mRNA expression[78]. Recent findings indicate that miRNAs play crucial roles in biological processes including cellular differentiation, proliferation and apoptosis[78,79]. Alterations in miRNA expression are described in a wide range of human diseases such as metabolic-, neurodevelopmental-, inflammatory-, cardiovascular- and liver diseases[80,81]. In addition, miRNAs have emerged as key regulators of lipid and glucose metabolism and play pivotal roles in the onset of obesity and obesity-related diseases by affecting status and functions of the adipose tissue, pancreas, liver and muscle[82].

Although the associations of obesity with EC and of miRNAs with EC are well documented, there is a scarcity of data concerning the role of miRNAs as a potential link between obesity and EC. Gaining a better understanding of the relationship between obesity and EC can provide new insights in obesity-related endometrial tumorigenesis.

Diagnosis of EC: miRNA as diagnostic biomarker

Since abnormal expression of miRNAs can contribute to the development of cancer including gynaecological cancer, they can be a candidate in either detecting or monitoring cancer treatment [81,83–87]. Currently, EC diagnosis is made by endometrial biopsy, which is an invasive and often uncomfortable procedure[88,89]. In addition, there is a large discordancy between pre- and postoperative tumour classification

following endometrial biopsy[90]. This can lead to either undertreatment or overtreatment with unnecessary surgical procedures and associated complications. Therefore, to improve correct preoperative classification, the identification of a novel diagnostic biomarker is needed. Novel diagnostic biomarkers should be non-invasive to improve management, patient care and acceptability of women presenting with abnormal bleeding.

MiRNAs are detectable and stable in a huge variety of bodily fluids including serum, urine, saliva and Pap smear[91]. Therefore, miRNAs are promising as non-invasive diagnostic biomarkers[92,93]. However, the diagnostic value of miRNA for EC is unknown.

Aims of this thesis

To improve understanding of the obesity-related endometrial carcinogenesis and prevent further excessive rise in the incidence of EC. Therefore, we aim to study:

- The relationship between adiposity distribution (body composition) in relation to outcomes in endometrial cancer
- The association between socioeconomic deprivation, obesity and survival in endometrial cancer
- The value of haematological parameters as indicators for comorbidity and body composition in endometrial cancer
- The link between miRNA expression levels and obesity in endometrial cancer
- The diagnostic value of miRNA expression in endometrial cancer

Outline of this thesis

In **Chapter 2**, body fat measurements are assessed with CT-scan and analysed in relationship to mortality in endometrial cancer.

In **Chapter 3**, the relationship between sarcopenia, obesity and outcomes in endometrial cancer is explored.

In **Chapter 4**, the association between socioeconomic deprivation and survival in endometrial cancer patients is outlined.

In **Chapter 5**, the link between socioeconomic deprivation, BMI and mortality in endometrial cancer is investigated.

In **Chapter 6**, haematological parameters as indicators for comorbidity and body composition in endometrial cancer are described and their relationship with survival is analysed.

In **Chapter 7**, the diagnostic value of miRNA expression in endometrial cancer is reviewed.

In **Chapter 8**, the miRNA expression patterns in tissue samples of endometrial cancer patients are determined and compared to benign endometrial tissue.

In **Chapter 9**, the diagnostic value of urinary miRNA expression in endometrial cancer is examined and the relationship between urinary miRNA expression and obesity is described.

In **Chapter 10**, findings are reviewed and future perspectives including impact on forthcoming research are discussed.

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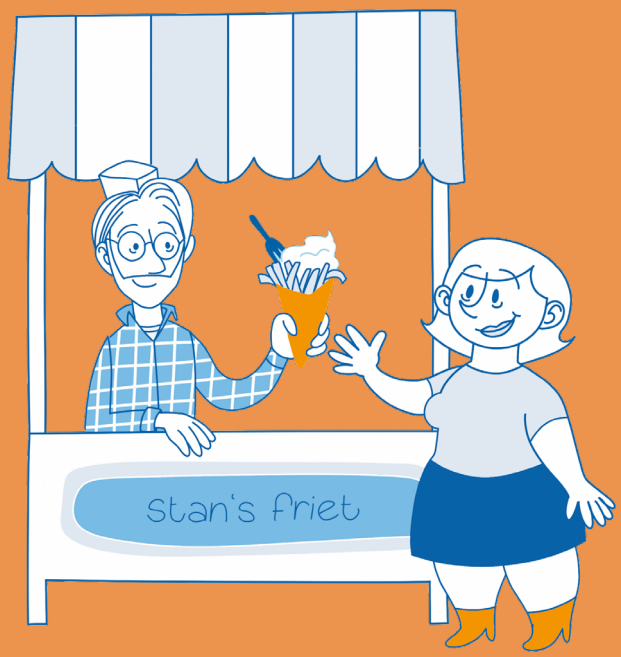
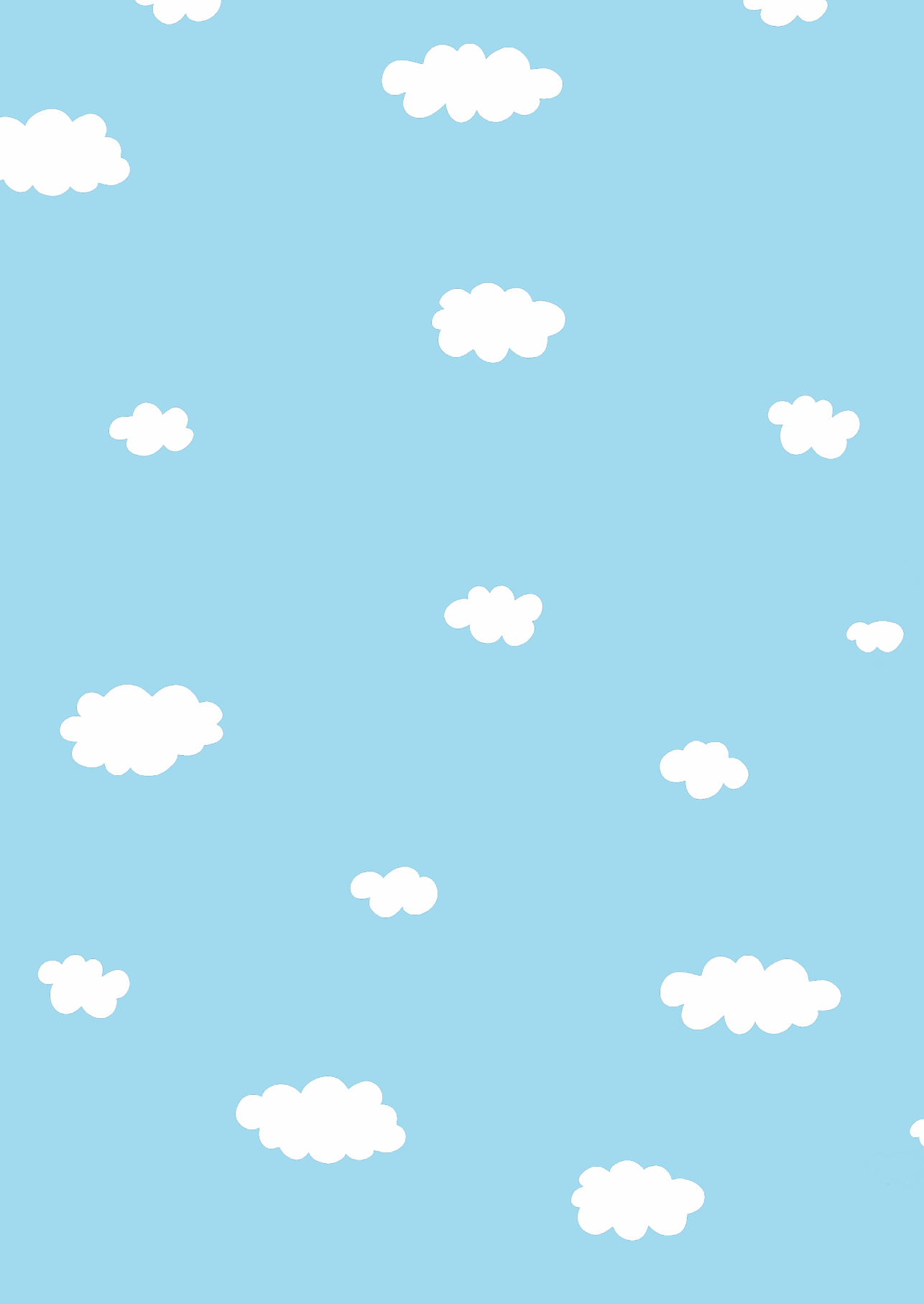
Part I

The impact of obesity on endometrial cancer development and progress





Chapter 2

Obesity and visceral fat: Survival impact in high-grade endometrial cancer

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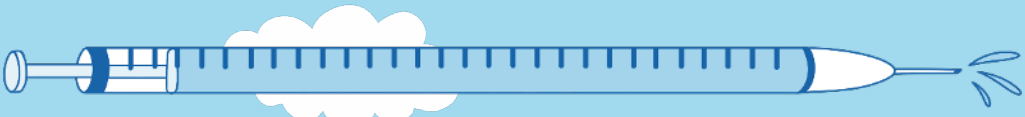


Abstract

Background
Obesity is an important risk factor for the development of endometrial cancer (EC). Recent data showed that body fat distribution might be more relevant than Body Mass Index (BMI). High visceral fat percentage was shown to be an independent predictor for survival in EC, but mainly included grade 1-2 EC.

Objective
To evaluate body fat distribution and its relation to outcome in high-grade EC.

Methods
Retrospective study in women diagnosed with high-grade EC between February 2006 and August 2017 at the Royal Cornwall Hospital who had abdominal CT-scan as part of routine diagnostic work-up. Subcutaneous abdominal fat volumes and visceral abdominal fat volumes were quantified based on CT-scan measurements, and visceral fat percentage calculated.

Results
A total of 176 patients with high-grade EC were included. The median age was 70 years and median BMI was 29.4 kg/m^2. The majority of patients had non-endometrioid endometrial cancer (NEEC; 62%). High visceral fat percentage was associated with poor overall- and disease-specific survival ($p = 0.006$ and $p = 0.026$, respectively) in NEEC patients, but not in high-grade endometrioid EC (EEC). The most frequent obesity comorbidities hypertension and diabetes mellitus were significantly associated with high BMI and high visceral fat percentage.

Conclusion
In high-grade EC, high visceral fat percentage was an independent predictor of poor survival only in NEEC. The strong correlation between high visceral fat and obesity-related comorbidities might be reflective of an unhealthy macroenvironment.

Introduction

In the United Kingdom (UK), 62% of women are either overweight or obese, with an increasing prevalence of obesity ranging from 15% in 1993 to 29% in 2019[1]. Obesity is strongly associated with the development of several serious medical conditions such as cardiovascular disease, type-2 diabetes and cancer including endometrial cancer (EC)[2]. Several mechanisms have been proposed to explain links between adiposity and EC including excess of endogenous sex steroid hormones, insulin resistance and chronic inflammation[3,4]. Although these mechanisms have been mainly attributed to endometrioid type EC (EEC), recent data illustrate that this is also relevant in non-endometrioid type EC (NEEC)[5].

Body Mass Index (BMI) is a well-recognised and widely used measure to classify adults into categories of underweight, normal weight, overweight and obese. However, there are limitations to the suitability of BMI as a measure of obesity as it does not incorporate metrics reflecting body composition[6,7]. Body fat is distributed into two main compartments with different metabolic characteristics: subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT)[8]. The accumulation of visceral fat, defined as fat around the viscera and inside the intra-abdominal parenchymal organs, is an independent risk factor in cardiovascular disease and is linked to poor outcomes in breast and ovarian cancer[9,10]. The gold standard for quantitative assessment of intra-abdominal adipose tissue is either by Computerized Tomography (CT) or Magnetic Resonance Imaging (MRI)[11,12].

The impact of obesity on survival in EC has been studied considerably, with studies suggesting worse outcomes in women with increased BMI, however the link between BMI and survival has not fully been established[17,18]. Therefore, accurate measures of body fat distribution in overweight and obese women would potentially allow us to assess the influence of obesity on survival and other health outcomes.

Evidence is scarce regarding the association between body fat distribution and outcomes including mortality, recurrence rates and surgical outcomes in EC. It has been hypothesised that high visceral fat rates are associated with worse outcomes, however previous studies have yielded conflicting results[19–21]. Furthermore, the largest study to date mainly included grade 1 or 2 EC[19].

Therefore, the aim of this study is to investigate the relationship between body fat distribution assessed by CT-scan in relation to overall and disease-specific survival in high-grade (grade 3) EC.

Materials and methods

Patients

Women diagnosed with high-grade primary EC between February 2006 and August 2017 at Royal Cornwall Hospital Trust (RCHT) Truro, Cornwall, United Kingdom were considered eligible for inclusion in this retrospective study. Inclusion criteria were women with grade 3 EEC or NEEC diagnosis who had CT-scan as part of standard management and exclusion criteria were women with grade 1 or 2 EC.

All included women had undergone contrast-enhanced abdominal CT (n = 174) or non-contrast CT (n = 2). CT-scans were conducted either preoperatively (n = 146) or within 6 weeks after surgery (n = 30). Indications for CT-scan postoperatively where either due to postoperative symptoms and/or complications or to establish baseline values for adjuvant treatment.

Women underwent standard treatment which included hysterectomy and bilateral salpingo-oophorectomy, when indicated followed by adjuvant therapy (radiotherapy and/or chemotherapy). Individualised management was given to women unfit for surgery or women who preferred non-surgical options, which included hormonal therapy, radiotherapy or chemotherapy. The majority of patients (84%) underwent surgical staging using the International Federation of Gynecology and Obstetrics (FIGO) staging criteria, accompanied by CT-scan as per local protocol[22]. For the 26 (15%) patients who did not undergo surgery, the FIGO stage was based on clinical examination and imaging results, and surgery was omitted due to advanced stage disease or patient preference. These patients were older and had more comorbidities when compared to the patients that underwent surgery. As the overall data were not affected, patients were included for analysis. Only 37 patients (21%) received either pelvic or para-aortic lymphadenectomy, since lymphadenectomy was only performed in high-risk EC patients with enlarged lymph nodes on imaging (CT-scan) as per British Gynaecological Cancer Society guidelines following the ASTEC trial[23,24].

Baseline characteristics were extracted from medical records, including patient- and tumour characteristics and CT report. BMI (weight in kg divided by height in meters squared) was based on measured weight and height during clinical visits within six weeks prior to surgery. Follow-up time varied from 27 months to 166 months, and patients were followed from the date of diagnosis until October 29th 2019, or death. Ethical and institutional approval was obtained for the study in accordance with the Declaration of Helsinki and was approved by the Health Research Authority (HRA) with reference number 19/SW/0111.

CT measurements

Abdominal CT images were analysed at the Mohn Medical Imaging and Visualization Centre (MMIV), Haukeland University Hospital, Bergen, Norway, using a semi-automated method for volumetric quantification of abdominal fat (iNtuition software program, TeraRecon Inc., San Mateo, CA, USA). Cross-sectional images were analysed consecutively from the upper right diaphragm to the level of vertebral body L5/S1, segmenting pixels with Hounsfield unit (HU) values corresponding to fat tissue (-195 to -45 HU), using a validated and reproducible method as previously described[26–29].

The segmented subcutaneous abdominal fat volume (SAV) and visceral abdominal fat volume (VAV) were visually verified by the operator (HD) and manually adjusted if necessary (illustrated in **Figure 1**).

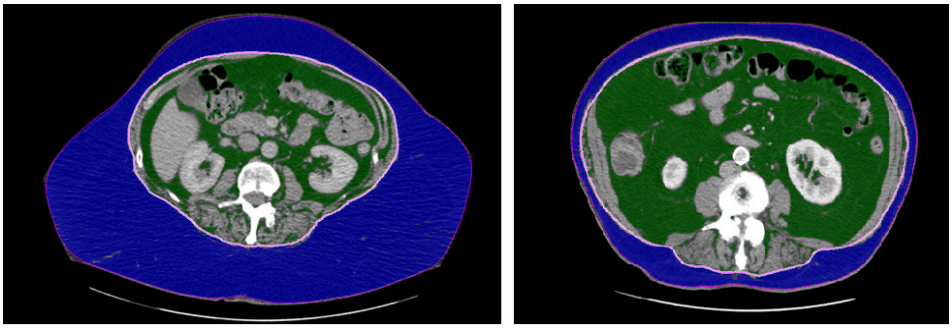


Figure 1. Cross-sectional abdominal CT image with segmentation of subcutanous (blue) and visceral (green) body fat for measurements of body fat distribution in two endometrial cancer patients.
***Left**: 70-year old patient (FIGO stage 1B, endometrioid subtype, BMI 39.1) with high SAV and low VAV%. Survival time 87 months (still alive)*
***Right**: 81-year old patient (FIGO stage IIIA, endometrioid subtype, BMI 26.5) with high VAV and high VAV%. Survival time 23 months (deceased)*

Internal validation was performed by a second operator (IH). VAV (cm^3) and SAV (cm^3) were estimated and the sum was considered to comprise the total abdominal fat volume (TAV, cm^3). The percentage of visceral out of total abdominal fat volume ([VAV/TAV]x100; VAV%) was also calculated. Waist circumference (cm) was measured at the level of vertebral body L3/L4.

Statistical analysis

For normally distributed variables, continuous variables were presented as medians and range and categorical variables presented as frequencies and proportions.

Nonparametric continuous data was compared using the Kruskal-Wallis test or Mann-Whitney U test, and Pearson Chi-square test was used for categorical data. Survival analysis were conducted using Kaplan-Meier curves and Cox Regression models while controlling for possible confounders (histology, FIGO stage, VAV%, BMI, age, hypertension and diabetes mellitus). The statistical significance of differences in outcome between the groups was assessed with the log-rank test. P-values less than 0.05 were considered significant for all tests. Data were analysed with IBM SPSS statistics version 26.0.

Results

Patient characteristics

In total, 176 women were included in this study. Median patient age was 70 years and median BMI was 29.4 kg/m^2 (**Table 1**). The majority of women had endometrioid subtype EC (38%), followed by serous (22%) and carcinosarcoma (20%). In total, 51% of women presented with FIGO stage I. Nearly the entire population was Caucasian (98%).

Table 1. Patient characteristics

Characteristics	Value
No. of patients	176
Age (years), median (range)	70 (58)
BMI (kg/m^2), median (range)	29.4 (39.4)
Overall survival (months), median (range)	42 (167)
BMI groups (%)	
< 25	39 (22)
25 – 30	54 (31)
> 30	77 (44)
Unknown	6 (3)
Ethnicity (%)	
Caucasian	173 (98)
Any other ethnic group	2 (1)
Unknown	1 (1)
Smoking (%)	
Yes	16 (9)
No	139 (79)
Ex-smoker	4 (2)
Unknown	17 (10)
Comorbidities (%)	
None	37 (21)
One	45 (26)
Two or more	92 (52)
Unknown	2 (1)
Diabetes Mellitus (%)	
Yes	22 (13)
No	154 (87)
Hypertension (%)	
Yes	90 (51)
No	86 (49)

table continues

Characteristics	Value
ECOG (%)	
0	92 (52)
1	49 (28)
≥2	23 (13)
Unknown	12 (7)
Histology (%)	
Endometrioid	66 (38)
Serous	38 (22)
Carcinosarcoma	35 (20)
Clear cell	15 (8)
Mixed tumours	8 (5)
Other	14 (7)
FIGO stage (%)	
I	90 (51)
II	10 (6)
III	36 (20)
IV	32 (18)
Unknown	8 (5)
Treatment modality (%)	
Laparoscopic hysterectomy + BSO	54 (31)
Laparotomy hysterectomy + BSO	91 (51)
Other type surgery	3 (2)
No surgery	26 (15)
Unknown	2 (1)
Lymphadenectomy (%)	
Yes	37 (25)
No	108 (72)
Unknown	5 (3)
Intraoperative complications (%)	
Yes	11 (7)
No	134 (90)
Unknown	5 (3)
Postoperative complications (%)	
Yes	36 (24)
No	110 (73)
Unknown	4 (2)
Adjuvant therapy (%)	
No	58 (33)
Yes	117 (67)
Unknown	1 (1)
Recurrence (%)	
Yes	41 (23)
No	113 (64)
Progression despite treatment	14 (8)
Unknown	8 (5)
Death (%)	
No	80 (45)
Yes	96 (55)
Cause of death (%)	
Endometrial cancer	70 (73)
Other	24 (25)
Unknown	2 (2)

Abbreviations: BMI = Body Mass Index, ECOG = Eastern Cooperative Oncology Group, FIGO = International Federation of Gynecology and Obstetrics

The association between BMI and CT-assessed obesity parameters and clinicopathological factors is shown in **Table 2**. Subtype EC (endometrioid versus non-endometrioid) was not associated with obesity parameters. However, patients with early FIGO stage had higher BMI (p = 0.036) than patients with advanced FIGO stage. Furthermore, elderly patients (aged ≥ 70 years) had higher VAV% compared to patients aged < 70 (p = 0.001). Two or more comorbidities were associated with all obesity parameters except for VAV.

Fat distribution and clinicopathological outcomes

Conversion from laparoscopy to laparotomy was more frequent in patients with high BMI (p = 0.004). In univariate analysis, none of the obesity parameters were associated with intraoperative complication rate. However, high WC (p = 0.027) and high VAV (p = 0.013) were associated with higher postoperative complication rates. In multivariate analysis, VAV (p = 0.039) was significantly associated with higher number of postoperative complications, but WC (p = 0.091) was not, data not shown (model adjusted for histological subtype, FIGO stage, age, hypertension and diabetes mellitus).

All obesity parameters were associated with the presence of hypertension and all obesity parameters with the exception of SAV were associated with diabetes mellitus (**Table 3**).

Table 2. BMI and CT estimated parameters in relation to clinicopathological factors

	N (%)	BMI	p	WC	p	TAV	p	VAV	p	SAV	p	VAV%	p
Histological subtype			0.66		0.64		0.88		0.64		0.28		0.97
Endometrioid	66 (38)	29.7		102		8506		2964		5842		34.0	
Non-endometrioid	110 (62)	28.9		98		8239		2827		4849		34.2	
FIGO stage			**0.036***		0.51		0.08		0.88		0.64		0.88
I + II	100 (60)	30.0		103		9113		2959		5740		33.9	
III + IV	68 (40)	27.9		97		7875		2873		5006		34.1	
Age (median)			0.88		0.88		0.17		0.45		0.17		**0.001***
< 70 years	82 (47)	29.3		99		9487		2660		6294		31.0	
≥ 70 years	94 (53)	29.4		99		7834		2945		4697		35.9	
Comorbidities			**<0.001***		**0.001***		**0.013***		0.10		**0.006***		**0.035***
None or one	82 (47)	27.6		96		7329		2626		4600		32.9	
Two or more	92 (53)	30.3		104		9268		3293		6085		35.5	
Intraoperative complications			0.21		0.49		0.52		0.20		0.52		0.52
Yes	11 (8)	32.6		104		9504		3618		5256		35.7	
No	134 (92)	29.7		99		8616		2861		6489		33.3	
Postoperative complications			0.11		**0.027***		0.08		**0.013***		0.33		0.18
Yes	36 (25)	32.6		104		9851		3660		6428		35.9	
No	110 (75)	28.8		98		8006		2647		4849		32.5	
Conversion to laparotomy			**0.004***		0.58		0.11		0.11		0.34		0.75
Yes	12 (18)	33.2		104		10485		3921		6891		35.6	
No	54 (82)	29.4		101		8879		2941		5088		34.1	
Recurrence			0.74		0.92		0.32		0.32		1.00		0.91
Yes	56 (33)	29.7		101		8157		2711		5346		33.8	
No	113 (67)	29.4		100		6557		2730		5304		33.8	

Abbreviations: BMI = Body Mass Index, FIGO = International Federation of Gynecology and Obstetrics, p: p-values, SAV = subcutaneous abdominal fat volume, TAV = total abdominal fat volume, VAV = visceral abdominal fat volume, VAV% = percentage of visceral out of total abdominal fat volume, WC = waist circumference
** Significant at p = 0.05 level*
Values are presented as medians

Table 3. Association of obesity parameters with obesity related comorbidities.

	BMI	WC	VAV	SAV	TAV	VAV%
DM						
Yes (N = 22)	32.4	110	4480	6861	11038	39.6
No (N = 154)	28.4	98	2689	4871	7833	33.3
p-value	**<0.001***	**0.003***	**0.001***	0.11	**0.003***	**0.001***
Hypertension						
Yes (N = 90)	30.8	104	3205	6054	9188	35.2
No (N = 86)	27.2	94	2287	4395	6597	32.6
p-value	**0.002***	**0.001***	**0.002***	**0.010***	**0.004***	**0.016***
DM + hypertension						
Yes (N = 17)	32.6	109	4322	6943	11294	36.8
No (N = 159)	28.6	98	2721	4937	7929	33.4
p-value	**0.002***	**0.011***	**0.002***	0.13	**0.011***	**0.002***

Abbreviations: BMI = Body Mass Index, DM = Diabetes Mellitus, SAV = subcutaneous abdominal fat volume, TAV = total abdominal fat volume, VAV = visceral abdominal fat volume, VAV% = percentage of visceral out of total abdominal fat volume, WC = waist circumference
** Significant at p < 0.05 level*
Values are presented as medians

Fat distribution and survival

High VAV% was significantly associated with reduced OS (p = 0.002) and DFS (p = 0.041) in the univariable Cox-model (**Table 4**).

Table 4. Univariate hazard ratios for OS and DSS and obesity parameters

	Overall survival				Disease-specific survival			
	N	Unadjusted HR	95% CI	p-value	N	Unadjusted HR	95% CI	p-value
BMI	170	1.02	0.99 – 1.05	0.22	170	1.00	0.99 – 1.06	0.19
WC	176	1.00	0.99 – 10.2	0.33	176	1.00	0.99 – 1.02	0.46
TAV	176	1.00	1.00 – 1.00	0.78	176	1.00	1.00 – 1.00	0.84
VAV	176	1.00	1.00 – 1.00	0.10	176	1.00	1.00 – 1.00	0.29
SAV	176	1.00	1.00 – 1.00	0.64	176	1.00	1.00 – 1.00	0.78
VAV%	176	1.04	1.01 – 1.06	**0.002***	176	1.03	1.00 – 1.06	**0.041***

Abbreviations: HR = hazard ratio, BMI = Body Mass Index, WC = waist circumference, TAV = total abdominal fat volume, VAV = visceral abdominal fat volume, SAV = subcutaneous abdominal fat volume, VAV% = percentage of visceral out of total abdominal fat volume.
** Significant at p < 0.05 level*

Survival curves are visualised in the Kaplan-Meier plots where patients with VAV% above median (34.1%) had significantly reduced OS (p = 0.024) but not DSS (p =

0.35) (**Figure 2A and B**). Mean overall survival time for patients with low VAV% was 88 months compared to 73 months for patients with high VAV% and mean DSS time was 96 months with low VAV% compared to 98 months in patients with high VAV%. Patients with higher VAV% did not have a higher risk for recurrent disease (p = 0.91).

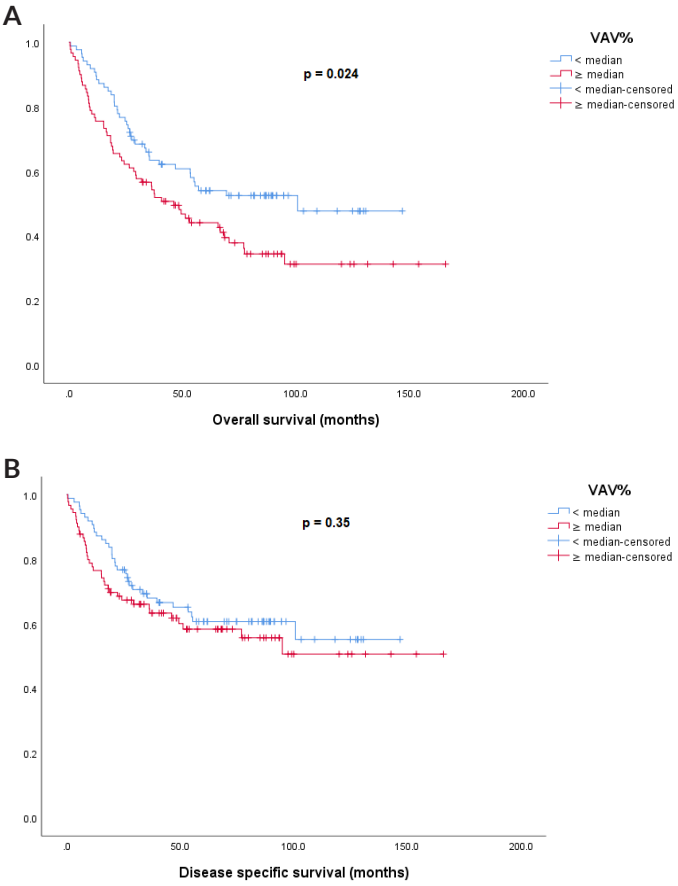


Figure 2. High VAV% is associated with worse overall survival, but not disease specific survival

A. *Kaplan Meier curve showing reduced OS in patients with high VAV% (median cut-off: 34.1%)*

B. *Kaplan Meier curve showing no difference in DSS in patients with high VAV%*

In multivariate Cox Proportional Hazards Regression model, histological subtype (p = 0.031), FIGO stage (p < 0.001), BMI (p = 0.007) and age (p = 0.006) were associated with OS after adjusting for confounders, but VAV% did not remain significant (p = 0.10) (**Table 5**). Similarly, histological subtype (p = 0.025), FIGO

stage (p < 0.001) and BMI (p = 0.017) were associated with DSS in multivariate analysis, but VAV% was not (p = 0.11).

Table 5. Prognostic impact of VAV% on OS and DSS (Cox proportional hazards regression model)

	N	Unadjusted HR	95% CI	p-value	Adjusted HR	95% CI	p-value
Overall survival							
Histological subtype (EEC vs NEEC)	176	0.59	0.38 – 0.92	**0.018***	0.59	0.36 – 0.95	**0.031***
FIGO stage	168	1.69	1.42 – 2.00	**<0.001***	1.76	1.47 – 2.10	**<0.001***
VAV%	176	1.04	1.01 – 1.06	**0.002***	1.03	1.00 – 1.06	0.10
BMI	170	1.02	0.99 – 1.05	0.22	1.05	1.01 – 1.08	**0.007***
Age	176	1.05	1.03 – 1.07	**<0.001***	1.03	1.01 – 1.06	**0.006***
Hypertension	176	1.65	1.09 – 2.48	**0.017***	0.98	0.60 – 1.59	0.92
DM	176	1.52	0.87 – 2.64	0.14	0.98	0.53 – 1.93	0.98
Disease specific survival							
Histological subtype (EEC vs NEEC)	176	0.52	0.31 – 0.88	**0.015***	0.52	0.29 – 0.92	**0.025***
FIGO stage	168	1.95	1.59 – 2.39	**<0.001***	2.05	1.66 – 2.53	**<0.001***
VAV%	176	1.03	1.01 – 1.06	**0.041***	1.03	0.99 – 1.07	0.11
BMI	170	1.02	0.99 – 1.06	0.19	1.05	1.01 – 1.09	**0.017***
Age	176	1.02	1.00 – 1.04	0.05	1.01	0.99 – 1.04	0.42
Hypertension	176	1.59	0.99 – 2.57	0.06	0.95	0.54 – 1.68	0.86
DM	176	1.32	0.68 – 2.59	0.42	0.97	0.45 – 2.08	0.94

Abbreviations: BMI = Body Mass Index, CI = Confidence Interval, EEC = endometrioid endometrial cancer, FIGO = International federation of Gynecology and obstetrics, HR = Hazard ratio, NEEC = non-endometrioid endometrial cancer, VAV% = Visceral fat percentage, DM = Diabetes Mellitus.
** Significant at p < 0.05 level*
Model adjusted for histology, FIGO stage, VAV%, BMI, age, hypertension and Diabetes Mellitus

However, sub-analysis in non-endometrioid endometrial cancer (NEEC) patients showed a significant correlation between high VAV% and OS (p = 0.006) and DSS (p = 0.026) in multivariate analysis (**Table 6**).

Survival curves are visualised in the Kaplan-Meier plots where NEEC patients with VAV% above median had significantly reduced OS (p = 0.009) but not DSS (p = 0.22) (**Figure 3A and B**). Moreover, BMI was associated with both OS (p = 0.002) and DSS (p = 0.026) and FIGO stage was also associated with OS (p < 0.001) and DSS (p < 0.001).

In endometrioid endometrial cancer (EEC) patients, only FIGO stage (p < 0.001 for OS and p < 0.001 for DSS) and age (p = 0.012 for OS and p = 0.048 for DSS) was associated with poorer survival and no association between survival and VAV% was seen (**Table 7**). Kaplan-Meier curves (**Figure 3C and D**) show no difference in survival for EEC patients with high VAV%.

Table 6. Prognostic impact of VAV% on OS and DSS in non-endometrioid endometrial cancer

	N	Unadjusted HR	95% CI	p-value	Adjusted HR	95% CI	p-value
Overall survival							
FIGO stage	107	1.81	1.47 – 2.24	**<0.001***	1.83	1.46 – 2.29	**<0.001***
VAV%	110	1.04	1.01 – 1.07	**0.003**	1.06	1.01 – 1.10	**0.006***
BMI	105	1.03	0.99 – 1.07	0.13	1.06	1.02 – 1.10	**0.002***
Age	110	1.04	1.02 – 1.07	**0.001***	1.01	0.98 – 1.04	0.41
Hypertension	110	1.44	0.88 – 2.37	0.15	0.80	0.46 – 1.41	0.44
DM	110	1.25	0.64 – 2.46	0.51	0.80	0.36 – 1.75	0.57
Disease specific survival							
FIGO stage	107	2.02	1.58 – 2.58	**<0.001***	2.13	1.63 – 2.78	**<0.001***
VAV%	110	1.03	1.00 – 1.06	0.09	1.05	1.01 – 1.10	**0.026***
BMI	105	1.03	0.98 – 1.07	0.24	1.05	1.01 – 1.11	**0.026***
Age	110	1.02	0.99 – 1.05	0.22	0.99	0.96 – 1.02	0.51
Hypertension	110	1.09	0.49 – 2.43	0.83	0.72	0.38 – 1.37	0.31
DM	110	1.32	0.75 – 2.31	0.34	0.88	0.35 – 2.22	0.78

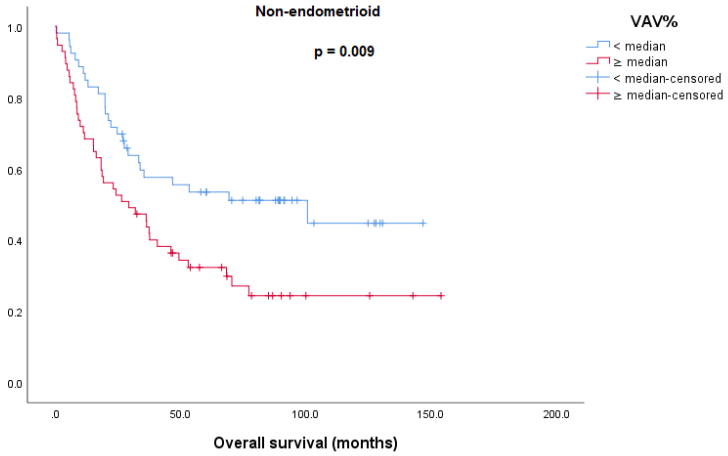
Abbreviations: BMI = Body Mass Index, CI = Confidence Interval, FIGO = International federation of Gynecology and obstetrics, HR = Hazard ratio, VAV% = Visceral fat percentage, DM = Diabetes Mellitus.
** Significant at p < 0.05 level*
Model adjusted for FIGO stage, VAV%, BMI, age, hypertension and Diabetes Mellitus

Table 7. Prognostic impact of VAV% on OS and DSS in endometrioid endometrial cancer

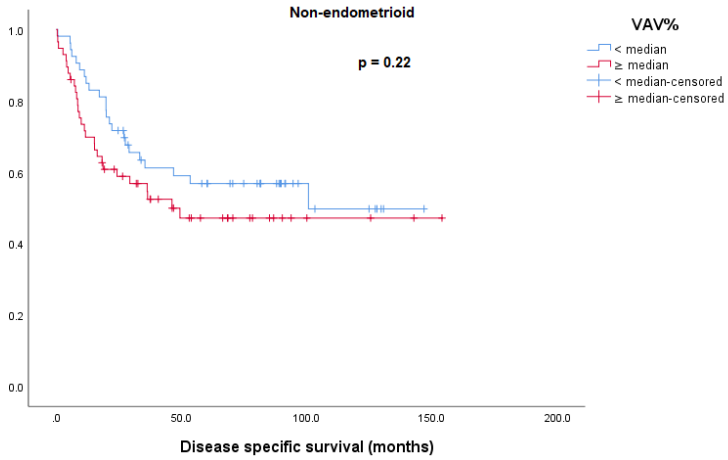
	N	Unadjusted HR	95% CI	p-value	Adjusted HR	95% CI	p-value
Overall survival							
FIGO stage	61	1.57	1.15 – 2.14	**0.004***	1.95	1.34 – 2.83	**<0.001***
VAV%	66	1.01	0.97 – 1.06	0.56	1.00	0.94 – 1.06	0.93
BMI	65	1.00	0.95 – 1.06	0.88	1.02	0.94 – 1.11	0.68
Age	66	1.05	1.02 – 1.09	**0.003***	1.05	1.01 – 1.10	**0.012***
Hypertension	66	2.04	0.82 – 5.07	0.13	1.72	0.54 – 5.52	0.36
DM	66	1.74	0.51 – 5.97	0.38	0.85	0.24 – 2.98	0.80
Disease specific survival							
FIGO stage	61	1.95	1.32 – 2.87	**0.001***	2.62	1.64 – 4.20	**<0.001***
VAV%	66	1.02	0.96 – 1.08	0.54	1.01	0.94 – 1.09	0.78
BMI	65	1.02	0.96 – 1.09	0.52	1.06	0.96 – 1.16	0.27
Age	66	1.03	0.99 – 1.07	0.15	1.05	1.00 – 1.11	**0.048***
Hypertension	66	2.04	0.82 – 5.07	0.13	1.87	0.50 – 6.97	0.35
DM	66	1.74	0.51 – 5.97	0.38	0.77	0.19 – 3.40	0.77

Abbreviations: BMI = Body Mass Index, CI = Confidence Interval, FIGO = International federation of Gynecology and obstetrics, HR = Hazard ratio, VAV% = Visceral fat percentage, DM = Diabetes Mellitus.
** Significant at p < 0.05 level*
Model adjusted for FIGO stage, VAV%, BMI, age, hypertension and Diabetes Mellitus

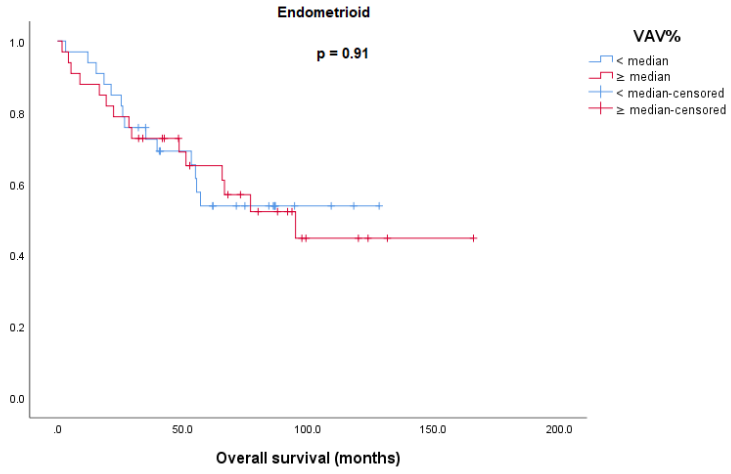
A


Non-endometrioid
p = 0.009
VAV%
< median
≥ median
< median-censored
≥ median-censored
1.0
0.8
0.6
0.4
0.2
0.0
.0
50.0
100.0
150.0
200.0
Overall survival (months)

B


Non-endometrioid
p = 0.22
VAV%
< median
≥ median
< median-censored
≥ median-censored
1.0
0.8
0.6
0.4
0.2
0.0
.0
50.0
100.0
150.0
200.0
Disease specific survival (months)

C


Endometrioid
p = 0.91
VAV%
< median
≥ median
< median-censored
≥ median-censored
1.0
0.8
0.6
0.4
0.2
0.0
.0
50.0
100.0
150.0
200.0
Overall survival (months)

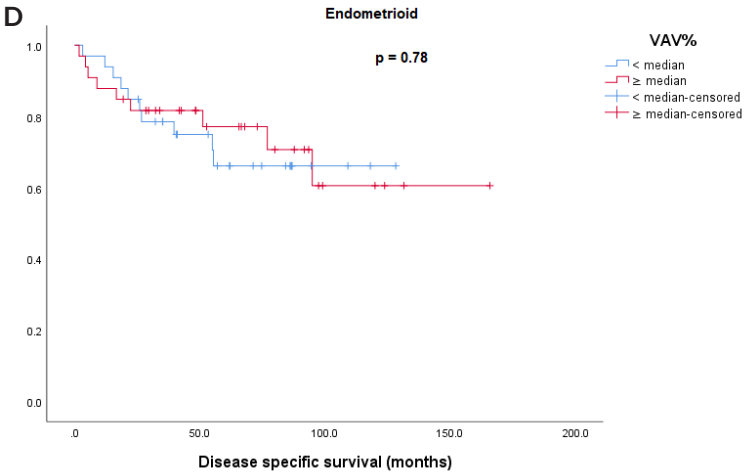


Figure 3. Differences in survival according to histological subtype
A. Kaplan Meier curve showing reduced OS in non-endometroid patients with high VAV%
B. Kaplan Meier curve showing no difference in DSS in non-endometrioid patients with high VAV%
C. Kaplan Meier curve showing no difference in OS in endometrioid patients with high VAV%
D. Kaplan Meier curve showing no difference in DSS in endometrioid patients with high VAV%

Discussion

To our knowledge, this is the largest study investigating body fat measurements on CT-scans in women with high-grade EC. In this study we found a relationship between high VAV% and poor OS and DSS specifically in NEEC. A study by Mauland et al. showed poor DSS in patients with higher VAV%, however included limited number of high-grade EC (32%); with 32 grade 3 endometrioid and 41 non-endometrioid[19]. They also found that increasing VAV% in EC patients was associated with older age, which is a well-known unfavourable prognostic factor in EC[19,30]. Furthermore, a smaller study by Nattenmüller in 54 EC patients showed no impact of VAV% on overall survival, however grade or subtype were not specified[20]. Previous literature has shown high VAV% to be associated with decreased OS in other cancer sites such as epithelial ovarian, oesophageal and pancreatic cancer, further supporting that increased visceral fat facilitates cancer progression due to obesity-induced inflammation and associated neutrophil infiltration which lead to the proliferation of adjacent tissues in the tumour microenvironment rather than reflecting an aggressive cancer phenotype[10,31,32].

The association found in this study between poor survival and high VAV% in NEEC patients is an interesting finding and supports the theory that not only EEC is related to obesity and outcome, but NEEC is also linked to obesity as supported by the data of Setiawan[5]. This is further highlighted by the fact that median BMI was similarly high for endometrioid patients versus non-endometrioid patients in our study.

It has been shown that with increasing age, fat distribution patterns change from a peripheral to a central fat deposition, although exact etiological pathways are unknown[33]. Our study has confirmed these results, finding higher VAV% in elderly patients, and even after adjusting for age, high VAV% was an independent predictor of poor survival in NEEC patients.

It is increasingly recognised that cancer progression and cancer-specific survival is not solely determined by the intrinsic characteristics of the tumour but also by host characteristics and in particular the systemic inflammatory response[34].

Furthermore, ageing is associated with an increase in systemic low-grade chronic inflammation as seen by raised levels of inflammatory markers such as IL-1 and C-reactive protein, which has been shown to contribute to morbidities and mortalities[35–37]. This can in turn lead to tissue degeneration and is associated with cancer induction and progression[34]. In addition, other age-related changes, obesity, comorbidities and tissue degradation also seem to drive the inflammatory response[38]. It has been hypothesised that systemic inflammatory response and hyperinsulinemia influence metabolic conditions such as diabetes and pre-existing comorbid disease as well as cancer in women with EC[39]. Therefore, the interrelationship between the host factors of age, BMI, comorbidities and the systemic inflammatory response are complex. In our study, we found a strong relationship between VAV% and the obesity-related comorbidities hypertension and diabetes mellitus, further supporting the concept that those comorbid conditions reflect an unhealthy macroenvironment. Patients with diabetes mellitus and/or hypertension are at increased risk for cancer progression which should be monitored in EC patients in order to reduce morbidity and mortality[40,41].

Previous trials have shown that EC patients with high BMI have unfavourable surgical outcomes[17]. In addition, in gastric and rectal cancer, patients with more visceral fat are more likely to develop postoperative complications[42–44]. In addition, Palomba et al. showed an association between increased visceral fat and greater risk of conversion to laparotomy in obese EC patients[46]. In this study, there was an increased risk of postoperative complications in patients with high visceral fat volumes. Furthermore, conversion from laparoscopy to laparotomy was more frequent in patients with high BMI, but there was no association with

fat distribution parameters. In our study, obesity parameters were not associated with increased risk of intraoperative complications, this is probably due to low rate of intraoperative complications (7%).

This study underlines the importance of adiposity and its impact on survival and outcomes in EC survivors. To improve visceral adiposity, several treatment programs have been suggested. Lifestyle interventions including calorie restriction and physical activities are described most frequently[47]. Specifically high intensity resistance training can slow the loss of muscle, while nutrition therapy improves visceral adiposity, as there is a strong association between energy intake and visceral fat accumulation[48]. Kwon et al. showed the effectiveness of a combined exercise regime to significantly reduce VAV, SAV and VAV% in healthy female adults[49]. Exercise interventions should however be individualised for EC patients because of age, comorbidities and disabilities.

This study has shown the importance of visceral fat (VAV%) as a potential obesity marker as a supplement to BMI in NEEC patients. CT based quantitative measurements of VAV using semi-automated software appears simple, accurate and minimally time-consuming, thus representing a feasible supplement to BMI for better prognostication by incorporating image-based obesity markers in EC patients. We propose this may be considered for inclusion in the future as part of standard work-up for patients newly diagnosed with high-grade EC.

Strengths and limitations

Strengths of our study include the large sample size of body fat measurements on CT-scans in high-grade EC patients. Furthermore, a highly reproducible and quantitative analysis of SAV and VAV could be performed due to usage of standardised fat measurements. Additionally, this study had a long follow-up time of over 10 years, in which a relatively high number of events (n = 96) was found.

This study has limitations due to its retrospective design including completeness of previously recorded data. Furthermore, BMI calculations (measured at clinical visit) and all CT-based estimates were only measured at one particular time-point and no data regarding possible changes in BMI and/or fat distribution were collected that could be taken into account. In addition, the field of view (FOV) for the CT-scans were set to assess intraabdominal tumour spread and therefore in some of the very obese patients, the entire SAV was not completely visualised, therefore potentially slightly underestimating the measured SAV in these patients. However, this was only the case in a very small percentage of the patients (5%) and in these it was perceived that the effect on the total SAV-measures was minimal; thus, this limitation seems to have only minimally influenced the findings in this study. Furthermore, only 21% of the patients underwent full staging procedure including

lymphadenectomy. However, the recurrence and survival rate of patients that underwent a complete surgical staging was comparable to those that were not properly staged, therefore we presume that this has not affected our results. In addition, there was no difference in EEC and NEEC with respect to surgical staging.

Conclusions and clinical implications

Obesity with increased visceral fat is important in predicting survival in NEEC patients. High visceral fat percentage was specifically associated with reduced overall and disease-specific survival in NEEC patients, but not in high-grade EEC patients. Clinicians need to be aware of those patients with obesity-related comorbidities, increased risk of postoperative complications and cancer progression. Specialised rehabilitation with training programs and nutritional therapy aiming at reducing their visceral fat volumes might be an important step to improve outcome in these NEEC patients.

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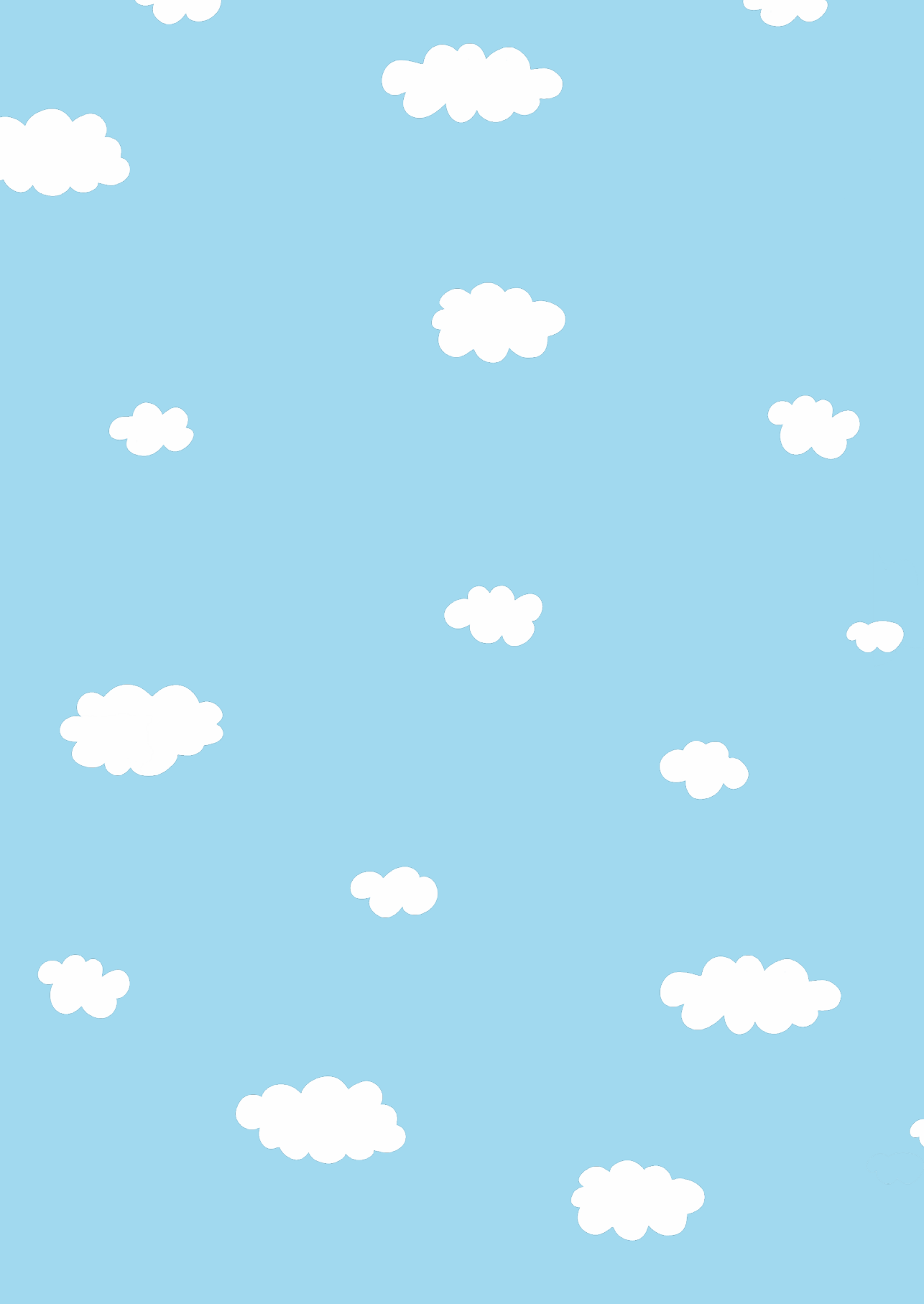

Chapter 3

The role of sarcopenic obesity in high-grade endometrial cancer

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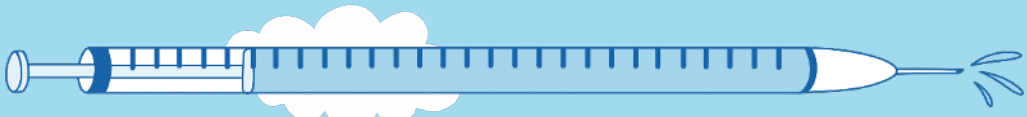

Abstract

Objective
To investigate the relationship between obesity and sarcopenia in relation to overall (OS) and disease-specific survival (DSS) in high-grade endometrial cancer (EC).

Methods
Retrospective study in women diagnosed with high-grade EC between February 2006 and August 2017 in the Royal Cornwall Hospital who had abdominal CT-scan as part of routine staging work-up. Sarcopenia was assessed by measuring psoas-, paraspinal- and abdominal wall muscles on CT and defined by skeletal muscle index ≤ 41 cm^2/m^2. Sarcopenic obesity was defined as sarcopenia combined with Body Mass Index (BMI) ≥ 30 kg/m^2.

Results
A total of 176 patients with median age of 70 years and median BMI of 29.4 kg/m^2 were included in the study. The majority of patients (38%) had endometrioid type histology. Sarcopenia was not associated with OS ($p = 0.95$) or DSS ($p = 0.55$). However, in multivariate analysis, sarcopenic obesity was associated with reduced OS in endometrioid endometrial cancer (EEC) patients ($p = 0.048$).

Conclusion
Sarcopenic obesity is associated with OS in high-grade EEC patients, while sarcopenia without obesity is not related to OS or DSS in high-grade EC. In NEEC, there is no association between sarcopenic obesity and survival.

Introduction

Endometrial cancer (EC) is the sixth most commonly occurring cancer in women worldwide, with over 380,000 new cases and nearly 90,000 deaths in 2018[1]. EC patients are often elderly with multiple comorbidities including obesity[2,3]. Due to the rise in obesity during the last decade, the incidence of EC has increased[4,5].

Although obesity in the general population is associated with an increased risk of death, there are conflicting reports about the relationship between obesity and mortality among individuals with cancer. In some studies, a survival benefit for overweight or even obese individuals has been demonstrated[6–10]. Furthermore, in EC, high Body Mass Index (BMI) and obesity is associated with low-grade, i.e. grade 1-2 endometrioid endometrial cancer (EEC), with relatively good overall survival[11]. This positive effect of high BMI on outcome is called the ''obesity paradox'', which suggests a potential protective effect of overweight or obesity. However, this reflects a complex relationship between obesity and EC. It has been hypothesised that reduced survival for people with normal BMI, compared with overweight, might be explained by loss of muscle mass[12].

Although BMI is a well-recognised and widely used measure to classify adults into categories of underweight, normal weight, overweight and obese, one major flaw and limitation is its inability to differentiate fat and muscle mass[13,14]. Some studies have therefore used waist-to-hip ratio (WHR) or waist circumference (WC); however these measures seem to lack the ability to differentiate subcutaneous from visceral fat deposition[15]. Therefore, in addition to BMI, there is a clear need for further specifying body composition incorporating muscle measurements. Currently, the gold standard for assessing body composition is Computerized Tomography (CT) or Magnetic Resonance Imaging (MRI)[16–18].

Since the median age group of women with EC is 61 years with 75-80% being postmenopausal, it is important to recognise that age is associated with alterations in body composition, with a decrease in muscle mass and strength[19,20]. Sarcopenia, a pathological decrease in muscle mass, is an objective measure of frailty that is associated with functional impairment and disability[21]. Furthermore, sarcopenia is associated with higher risk of cancer recurrence, reduced overall- and cancer-specific mortality, surgical complications and treatment-related toxicities[22].

Sarcopenia has been shown to have a strong mortality risk in all BMI groups, but mainly in obesity[23]. This is a condition called sarcopenic obesity and is associated with worse prognosis in several chronic diseases and various cancers[24–26].

The relationship between sarcopenia, obesity and outcome in EC patients is

unclear. Previous studies have shown sarcopenia to impact recurrence-free survival, but not overall survival in EC[27,28]. However, the interaction of obesity and sarcopenia in EC has not yet been studied. Furthermore, the largest study to date mainly included grade 1-2 EEC[28].

Therefore, the aim of this study is to investigate the relationship between obesity and sarcopenia in relation to overall- and disease-specific survival as measured by CT in high-grade EC patients.

Materials and methods

In this retrospective study, women diagnosed with primary EC between February 2006 and August 2017 at Royal Cornwall Hospital Trust (RCHT) Truro, Cornwall, United Kingdom were considered eligible for inclusion. Inclusion criteria were women diagnosed with primary EC, histology either endometrioid grade 3 or non-endometrioid carcinoma and ≥ 18 years of age, who had CT-scan as part of standard staging workup. Exclusion criteria were women with grade 1 or 2 EEC.
In total, 176 women were included, and all included women had undergone contrast-enhanced abdominal CT (n = 174) or non-contrast CT (n = 2). CT-scans were conducted either preoperatively or at time of diagnosis (n = 146) or within 6 weeks after surgery (n = 30). Indications for CT-scan postoperatively were either due to postoperative symptoms or complications or to establish baseline staging parameters before adjuvant treatment.

Women underwent standard treatment which included hysterectomy and bilateral salpingo-oophorectomy (BSO), followed by adjuvant therapy (radiotherapy and/or chemotherapy) when indicated. Individualised management was given to women unfit for surgery or women who preferred non-surgical options, which included hormonal therapy, radiotherapy or chemotherapy. The majority of patients (84%) underwent surgical staging using the International Federation of Gynecology and Obstetrics (FIGO) staging criteria, accompanied by CT-scan as per local protocol[29]. Staging procedure included para-aortic and/or pelvic lymph node dissection performed by gynaecological oncologist. For the 26 (15%) patients who did not undergo surgery, the FIGO stage was based on clinical examination and imaging results. Reasons surgery was omitted included advanced stage disease, patient unfit for surgery or patient preference. These patients were older and had more comorbidities when compared to the patients that underwent surgery; however, as the overall data were not affected, patients were included for analysis. Following the ASTEC trial, in most UK centres lymphadenectomy is exclusively performed in high-risk EC patients with enlarged lymph nodes on imaging by CT-scan[30]. Therefore, only 37 patients (21%) underwent either pelvic or para-aortic lymphadenectomy.

For every case, a formal review of the pathology slides was performed at the Multidisciplinary Team Meeting (MDT). This allows for two pathologist to review slides independently of each other.

Ethical and institutional approval was obtained for the study in accordance with the Declaration of Helsinki and was approved by the Health Research Authority (HRA) with reference number 19/SW/0111. It was deemed informed consent was not needed due to the retrospective nature of this study.

Baseline characteristics were obtained from patient records, including patient- and tumour characteristics. BMI (weight in kg divided by height in meters squared) was based on measured weight and height during clinical visits within six weeks prior to surgery. Follow-up time varied from 27 months to 166 months and patients were followed from the date of diagnosis until October 29th 2019 or death.

Abdominal CT images were analysed at the Mohn Medical Imaging and Visualization Centre (MMIV), Haukeland University Hospital, Bergen, Norway, using a semi-automated method for volumetric quantification of abdominal fat (iNtuition software program, TeraRecon Inc., San Mateo, CA, USA).

Sarcopenia markers were measured on cross-sectional image on which both vertebral transverse processes of the third lumbar vertebra (L3) were most clearly depicted. L3 was chosen as the standard landmark according to previously published studies[16,31]. The selected image had to be of sufficient quality for valid muscle segmentation analysis, meaning no imaging artefacts inducing difficulties in valid muscle segmentation[32]. Based on these criteria, no patients were excluded for analysis. The relevant muscles were identified based on anatomical localisation and comprised the psoas, paraspinal and abdominal wall muscles, which have been previously used to determine muscle mass and sarcopenia[16]. The iNtuition software was used to manually delineate muscular tissue using threshold values of HU units of -29 to + 150 by the operator (HD) (**Figure 1**)[32].

Internal validation was performed by a second operator (IH). The software program computed skeletal muscle area (SMA; cm^2) by summing cross-sectional muscle areas. Skeletal muscle index (SMI; cm^2/m^2) was calculated by correcting SMA for height: SMA (cm^2)/height $(m)^2$. Sarcopenia was defined as SMI < 41 cm^2/m^2 according to threshold values described previously[33]. Sarcopenic obesity was defined as sarcopenia plus BMI ≥ 30 kg/m^2.

With a background prevalence of sarcopenic obesity of 7% in the general population and 15% in cancer patients, we performed a sample size calculation (α = 0.05 and power of 80%). The sample size was calculated to be 100 patients[34–36].

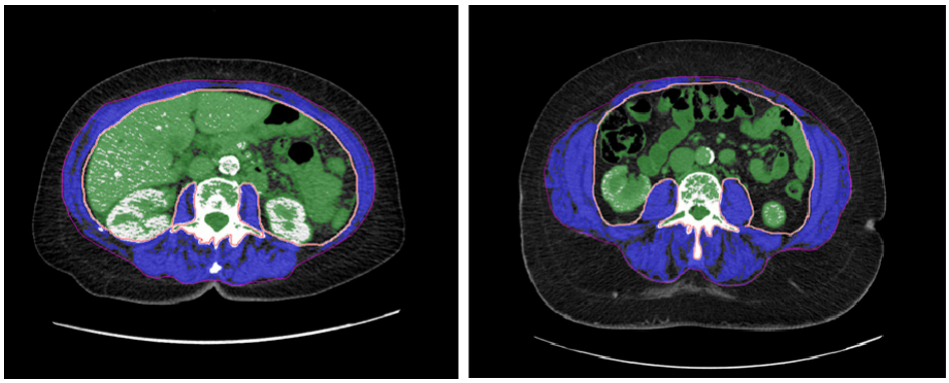

Figure 1. Cross-sectional abdominal CT image at the level of the vertebral transverse processes of L3 with segmentation of skeletal muscles (blue), viscera in green.
***Left**: 72-year old patient (FIGO stage 2, serous subtype, BMI 38) with low skeletal muscle area indicating sarcopenia. Survival time 11 months (deceased)*
***Right**: 77-year old patient (FIGO stage IA, endometrioid subtype, BMI 22) with high skeletal muscle area. Survival time 62 months (still alive)*

For variables having normal distribution, continuous variables were presented as medians and range and categorical variables presented as frequencies and proportions. Nonparametric continuous data was compared using the Kruskal-Wallis test or Mann-Whitney U test, and Pearson Chi-square test was used for categorical data. Survival data was analysed using Kaplan-Meier curves and Cox Regression models while controlling for possible confounders. The statistical significance of differences in outcome between the groups was assessed with the log-rank test. P-values less than 0.05 were considered significant for all tests. Data were analysed with IBM SPSS statistics version 26.0.

Results

In total, 176 women were included in this study. Median patient age was 70 years and median BMI was 29.4 kg/m^2 (**Table 1**). The majority of women had endometrioid subtype EC (38%), followed by serous carcinoma (22%) and carcinosarcoma (20%). Nearly the entire population was Caucasian (98%). Out of these 176 women, 61 (35%) were sarcopenic and 17 (10%) met the criteria for sarcopenic obesity.

Sarcopenic patients had similar age as patients without sarcopenia but sarcopenic patients had lower BMI (p = 0.002) and higher FIGO stage at diagnosis (p = 0.031) (**Table 2**). However, sarcopenia was not associated with disease recurrence (p = 0.27) or overall mortality (p = 0.76).

Table 1. Patient characteristics

Characteristics	Total population	Sarcopenia	Sarcopenic obesity
No. of patients	176	61	17
Age (years), median (range)	70 (58)	72 (49)	72 (23)
BMI (kg/m²), median (range)	29.4 (39.4)	25.7 (22.2)	32.6 (11.4)
Overall survival (months), median (range)	42 (167)	51 (131)	40 (90)
BMI groups (%)			
< 25	39 (22)	25 (41)	0 (0)
25 – 30	54 (31)	18 (30)	0 (0)
> 30	77 (44)	18 (30)	17 (100)
Unknown	6 (3)	0 (0)	0 (0)
Ethnicity (%)			
Caucasian	173 (98)	60 (98)	16 (94)
Any other ethnic group	2 (1)	0 (0)	0 (0)
Unknown	1 (1)	1 (2)	1 (6)
Smoking (%)			
Yes	16 (9)	6 (10)	0 (0)
No	139 (79)	48 (79)	17 (100)
Ex-smoker	4 (2)	2 (3)	0 (0)
Unknown	17 (10)	5 (8)	0 (0)
Comorbidities (%)			
None	37 (21)	12 (20)	2 (12)
One	45 (26)	18 (30)	3 (18)
Two or more	92 (52)	31 (51)	12 (71)
Unknown	2 (1)	0 (0)	0 (0)
Diabetes Mellitus (%)			
Yes	22 (13)	5 (8)	3 (18)
No	154 (87)	56 (92)	14 (82)
Hypertension (%)			
Yes	90 (51)	28 (46)	12 (71)
No	86 (49)	33 (54)	5 (29)
ECOG (%)			
0	92 (52)	30 (49)	7 (41)
1	49 (28)	18 (30)	7 (41)
≥2	23 (13)	5 (8)	2 (12)
Unknown	12 (7)	2 (3)	1 (6)
Histology (%)			
Endometrioid	66 (38)	22 (36)	4 (24)
Serous	38 (22)	15 (25)	4 (24)
Carcinosarcoma	35 (20)	13 (21)	4 (24)
Clear cell	15 (9)	7 (12)	2 (12)
Mixed tumours	8 (5)	1 (2)	1 (6)
Other	14 (6)	1 (2)	2 (12)
Unknown	0 (0)	2 (3)	0 (0)
FIGO stage (%)			
I	90 (51)	29 (48)	10 (59)
II	10 (6)	5 (8)	1 (6)
III	36 (21)	8 (13)	2 (12)
IV	32 (18)	16 (26)	4 (24)
Unknown	8 (5)	3 (5)	0 (0)

table continues

Characteristics	Total population	Sarcopenia	Sarcopenic obesity
Treatment modality (%)			
Laparoscopic hysterectomy + BSO	54 (31)	20 (33)	8 (47)
Laparotomy hysterectomy + BSO	91 (52)	28 (46)	7 (41)
Other type surgery	3 (2)	0 (0)	0 (0)
No surgery	26 (15)	11 (18)	2 (12)
Unknown	2 (1)	2 (3)	0 (0)
Lymphadenectomy (%)			
Yes	37 (25)	13 (26)	10 (67)
No	108 (72)	34 (68)	5 (33)
Unknown	5 (3)	3 (6)	0 (0)
Intraoperative complications (%)			
Yes	11 (7)	1 (2)	1 (7)
No	134 (90)	46 (92)	14 (93)
Unknown	5 (3)	3 (6)	0 (0)
Postoperative complications (%)			
Yes	36 (24)	8 (16)	12 (80)
No	110 (74)	39 (78)	3 (20)
Unknown	4 (2)	3 (6)	0 (0)
Adjuvant therapy (%)			
No	58 (33)	18 (35)	5 (29)
Yes	117 (67)	32 (63)	12 (71)
Unknown	1 (1)	1 (2)	0 (0)
Recurrence (%)			
Yes	41 (23)	13 (21)	5 (29)
No	113 (64)	40 (66)	11 (65)
Progression despite treatment	14 (8)	2 (3)	1 (6)
Unknown	8 (5)	6 (10)	0 (0)
Death (%)			
No	80 (46)	30 (49)	8 (47)
Yes	96 (54)	31 (51)	9 (53)
Cause of death (%)			
Endometrial cancer	70 (73)	25 (41)	8 (89)
Other	24 (15)	6 (10)	1 (11)
Unknown	2 (1)	0 (0)	0 (0)

Abbreviations: BMI = Body Mass Index, BSO = bilateral salpingo-oophorectomy, ECOG = Eastern Cooperative Oncology Group, FIGO = International Federation of Gynecology and Obstetrics

Sarcopenic obesity patients had similar baseline characteristics compared to patients without sarcopenic obesity, with the exception of BMI (median BMI 32.6 for sarcopenic obese patients and 28.3 for non-sarcopenic obese patients, $p < 0.001$, **Supplementary Table 1**).

Sarcopenia was not associated with OS ($p = 0.95$) or DSS ($p = 0.55$) in EC patients (**Figure 2**), with median overall survival time 69 months (95% CI 44 – 95 months) for sarcopenic patients and 55 months (95% CI 18 – 93 months) for non-sarcopenia patients.

Table 2. Differences in clinicopathological factors between patients with and without sarcopenia (univariate analysis)

Patient characteristics	Sarcopenia (N = 61)	No sarcopenia (N = 107)	p-value
Age, median (IQR)	72 (15)	69 (16)	0.37
BMI, median (IQR)	25.7 (6.0)	31.4 (8.9)	**0.002***
Histology (%)			0.60
Endometrioid	22 (36)	43 (40)	
Non-endometrioid	39 (64)	64 (60)	
Stage (%)			**0.031***
I	29 (48)	60 (56)	
II	5 (8)	4 (4)	
III	8 (13)	26 (24)	
IV	16 (26)	13 (12)	
Comorbidities (%)			0.65
0	12 (20)	25 (23)	
1	18 (30)	25 (23)	
2+	31 (51)	57 (53)	
Diabetes Mellitus (%)			0.20
Yes	5 (8)	16 (15)	
No	56 (92)	91 (85)	
Hypertension (%)			0.42
Yes	28 (46)	56 (52)	
No	33 (54)	51 (48)	
Recurrence (%)			0.27
Yes	15 (25)	38 (36)	
No	40 (66)	68 (64)	
Death (%)			0.76
Yes	31 (51)	57 (53)	
No	30 (49)	50 (47)	
Cause of death (%)			0.70
Endometrial cancer	25 (81)	39 (70)	
Other	6 (19)	17 (30)	

Abbreviations: BMI = Body Mass Index, ECOG = Eastern Cooperative Oncology Group, IQR = interquartile range, SMI = skeletal muscle index
Numbers don't add up due to missing values
** Significant at p < 0.05 level*

In multivariate analysis, sarcopenic obesity (p = 0.048), FIGO stage (p = 0.005) and age (p = 0.015) were associated with OS in high-grade EEC patients (**Table 3**).

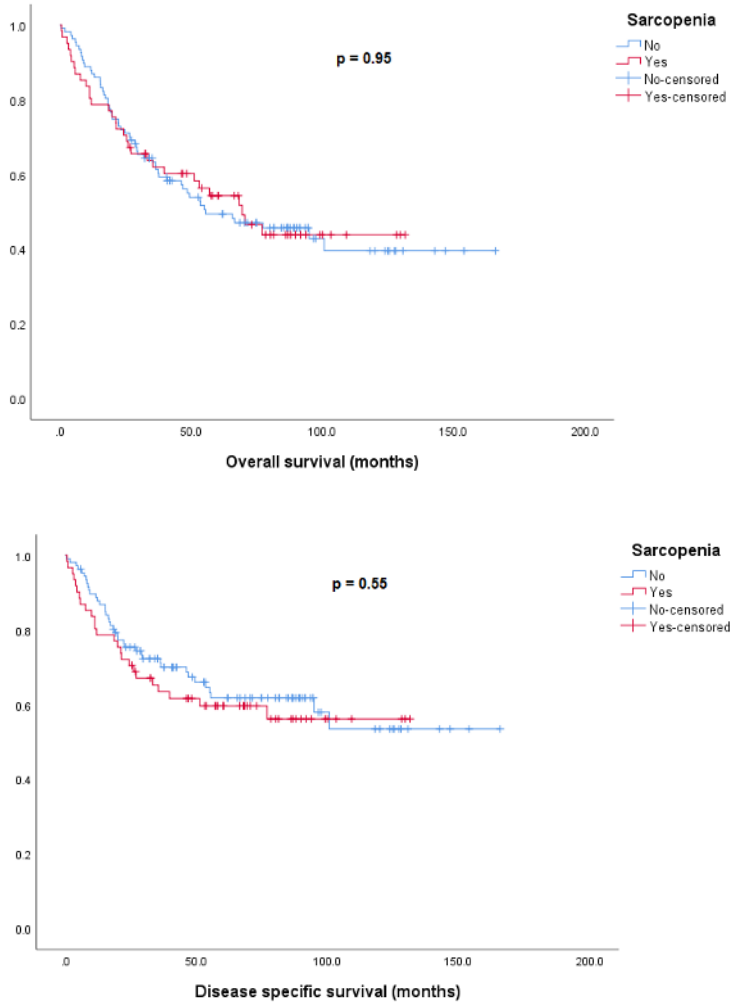

Figure 2. Sarcopenia is not associated with survival in high-grade endometrial cancer
A. Kaplan Meier curve showing similar OS for patients with sarcopenia
B. Kaplan Meier curve showing similar DSS for patients with sarcopenia

In NEEC patients, FIGO stage ($p < 0.001$), BMI ($p = 0.007$) and age ($p = 0.024$) were associated with OS (**Supplementary Table 2**). For DSS, only FIGO stage ($p < 0.001$) and BMI ($p = 0.042$) remained significantly associated.

Table 3. Prognostic impact of sarcopenic obesity on OS and DSS for high-grade endometrioid endometrial cancer

	N	Unadjusted HR	95% CI	p-value	Adjusted HR	95% CI	p-value
Overall survival							
FIGO stage	61	1.57	1.15 – 2.14	**0.004***	1.69	1.17 – 2.43	**0.005***
BMI	66	1.00	0.95 – 1.06	0.88	1.01	0.93 – 1.10	0.76
Age	65	1.05	1.02 – 1.09	**0.003***	1.06	1.01 – 1.12	**0.015***
Comorbidities (2+)	66	3.24	1.38 – 7.60	**0.007***	1.22	0.35 – 4.24	0.78
Sarcopenic obesity	65	3.09	0.93 – 10.3	0.07	4.22	1.02 – 17.6	**0.048***
Disease specific survival							
FIGO stage	61	1.95	1.32 – 2.87	**<0.001***	2.17	1.34 – 3.50	**0.002***
BMI	65	1.02	0.96 – 1.09	0.52	1.05	0.94 – 1.16	0.41
Age	66	1.03	0.99 – 1.07	0.15	1.06	0.99 – 1.13	0.10
Comorbidities (2+)	66	3.71	1.23 – 11.20	**0.020***	1.56	0.30 – 8.14	0.60
Sarcopenic obesity	65	4.75	1.37 – 16.5	**0.014***	3.15	0.67 – 14.78	0.15

Abbreviations: BMI = Body Mass Index, CI = Confidence Interval, FIGO = International federation of Gynecology and obstetrics, HR = Hazard ratio.
** Significant at p < 0.05 level*

Discussion

To our knowledge, this is the first study assessing sarcopenia and sarcopenic obesity in high-grade EC. We showed no association between sarcopenia and OS or DSS in high-grade EC. However, an association was found between sarcopenic obesity and OS in high-grade EEC.

Previously, a study by Kuroki et al. showed sarcopenia to have a negative impact on recurrence-free survival, but not on overall survival in 122 EC patients[28]. However, they only included 49 patients with grade 3 disease and only 10% of women had NEEC tumours. Furthermore, a study by Nattenmüller et al. in 54 EC patients showed no impact of sarcopenia on OS[27]. Grade or subtype was not specified in this study. However, in both studies, obesity was not taken into account.

Increased mortality in sarcopenic patients has been reported for all BMI categories but is predominantly strong in obese patients[23]. The prevalence of sarcopenic obesity is increasing in elderly adults including adults with cancer[26,34]. Prado et al. showed sarcopenic obesity to be associated with poorer functional status and to be an independent predictor of survival in patients with respiratory- and gastrointestinal tumours[25].

Multiple factors are responsible for body composition alterations occurring during ageing. Body fat increases with age, peaking at about 60-75 years of age and thereafter decreasing[35,36]. Furthermore, muscle mass and strength start to decline progressively around the age of 30 years with a more pronounced loss after the age of 60[37]. In addition, visceral fat tends to increase, while subcutaneous fat declines[38]. Furthermore, fatty infiltration of skeletal muscle (myosteatosis) is an important component of aging and frailty[39]. The increase in body weight and fat are due to a progressive decline in total energy expenditure resulting from decreased physical activity levels and reduced basal metabolic rate[40]. Moreover, aging is associated with hormonal changes, a pro-inflammatory state, malnutrition and loss of alpha-motor units in the central nervous system, all accelerating the loss of muscle mass and strength[41,42]. These hormonal changes are mainly seen during and after menopause, in which lack of oestrogen is responsible for a decline in muscle mass. Oestrogens are necessary for satellite cell maintenance and function, which are essential for skeletal muscle regeneration[43,44].

Although it is generally believed that EEC is highly linked to obesity and NEEC have a different mechanistic background, recent data illustrate that NEEC are also linked to obesity[45]. In this study we found evidence for an association between poor survival and sarcopenic obesity in high-grade EEC, whereas no association was seen in NEEC. This raises questions about the different pathophysiology between high-grade EEC and NEEC and further emphasises the current incomplete understanding of the obesity-related endometrial carcinogenesis.

This study underlines the importance of adiposity and muscle loss and its impact on survival in EC patients. Several treatment programs have been described to improve adiposity and sarcopenia including life style changes with calorie restrictions and physical activities[34]. High intensity resistance training can slow the loss of muscle and can be valuable for posttreatment rehabilitation[46]. Dieli-Conwright et al. showed combined resistance and aerobic exercises effectively reduced sarcopenic obesity and features of metabolic syndrome in overweight and obese breast cancer survivors[47]. A recent systematic review in adults with sarcopenic obesity showed the importance of resistance exercise, while nutritional intervention with low-calorie high-protein diet decreased fat mass but did not improve physical performance[48]. Exercise treatments should however be individualised for EC patients with sarcopenic obesity because of age, comorbidities and disabilities.

This study has shown the importance of sarcopenia as a poor prognostic factor in high-grade EEC. CT based quantitative measurements of muscle mass using semi-automated software appears simple, accurate and minimally time-consuming and could therefore be used in the future for better prognostication in EEC patients.

Strengths and limitations

Strengths of our study include the largest study to date of sarcopenia measurements on CT-scans in EC patients. In addition, this study included a heterogenous group of EC patients with different histology and FIGO stage. Additionally, this study had a long follow-up time of over 10 years, in which a relatively high number of events (n = 96) was found.

The main limitation of this study was BMI calculations and all CT-based estimates were only measured at one particular time-point and no data regarding possible changes in BMI were collected. Furthermore, only 21% of the patients underwent full staging procedure including lymphadenectomy. However, the recurrence- and survival rate of patients that underwent a complete surgical staging was comparable to those that were not fully surgically staged, therefore we believe that this has not affected our results. In addition, there was no difference in EEC and NEEC with respect to surgical staging.

Conclusion

In our study, sarcopenia was not associated with survival in high-grade EC, however sarcopenic obesity seems an independent negative prognostic factor in high-grade EEC. Further studies are needed to investigate the role of sarcopenia in EC. Specialised rehabilitation with training programs aiming at increasing muscle mass could be an important step to improve outcome in these patients.

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Supplemental material

Supplementary Table 1. Differences in clinicopathological factors between patients with and without sarcopenic obesity (univariate analysis)

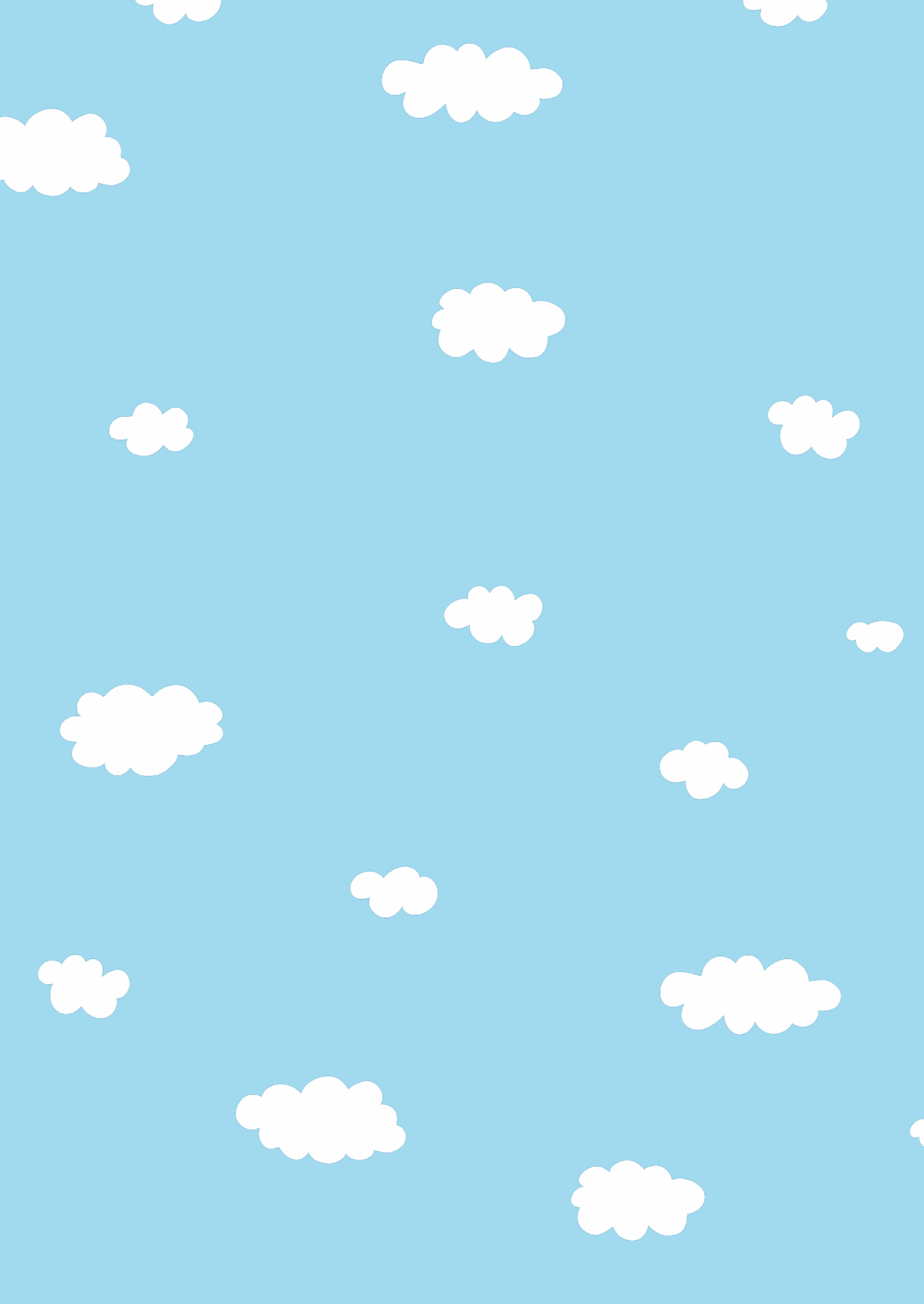
Patient characteristics	Sarcopenic obesity (N = 17)	No sarcopenic obesity (N = 151)	p-value
Age, median (IQR)	72 (9)	70 (17)	0.80
BMI, median (IQR)	32.6 (4.7)	28.3 (8.5)	**< 0.001***
Histology (%)			0.18
Endometrioid	4 (24)	61 (40)	
Non-endometrioid	13 (77)	90 (60)	
Stage (%)			0.76
I	10 (59)	79 (52)	
II	1 (6)	8 (5)	
III	2 (12)	32 (21)	
IV	4 (24)	25 (17)	
Comorbidities (%)			0.28
0	2 (12)	35 (23)	
1	3 (18)	40 (27)	
2+	12 (71)	76 (50)	
Diabetes Mellitus (%)			0.50
Yes	3 (18)	18 (12)	
No	14 (82)	113 (88)	
Hypertension (%)			0.07
Yes	12 (71)	72 (48)	
No	5 (29)	79 (52)	
Recurrence (%)			0.88
Yes	5 (29)	48 (32)	
No	11 (65)	97 (64)	
Death (%)			0.96
Yes	9 (53)	72 (48)	
No	8 (47)	79 (52)	
Cause of death (%)			0.69
Endometrial cancer	8 (89)	56 (72)	
Other	1 (11)	22 (28)	

Abbreviations: BMI = Body Mass Index, IQR = interquartile range.
Numbers don't add up due to missing values
** Significant at p < 0.05 level*

Supplementary Table 2. Prognostic impact of sarcopenic obesity on OS and DSS for non-endometrioid endometrial cancer

Overall survival							
	N	**Unadjusted HR**	**95% CI**	**p-value**	**Adjusted HR**	**95% CI**	**p-value**
FIGO stage	107	1.81	1.47 – 2.24	**< 0.001***	1.70	1.36 – 2.14	**< 0.001***
BMI	105	1.03	0.99 – 1.07	0.13	1.05	1.01 – 1.09	**0.007***
Age	110	1.04	1.02 – 1.07	**0.001**	1.03	1.00 – 1.06	**0.024***
Comorbidities (2+)	110	1.00	1.00 – 1.00	**< 0.001***	0.73	0.40 – 1.33	0.30
Sarcopenic obesity	103	0.68	0.29 – 1.58	0.37	0.78	0.32 – 1.89	0.58
Disease specific survival							
FIGO stage	107	2.02	1.58 – 2.58	**< 0.001***	2.01	1.54 – 2.62	**< 0.001***
BMI	105	1.03	0.98 – 1.07	0.24	1.05	1.00 – 1.09	**0.042***
Age	110	1.02	0.99 – 1.05	0.22	1.01	0.98 – 1.04	0.57
Comorbidities (2+)	110	1.00	1.00 – 1.00	**< 0.001***	0.53	0.28 – 1.08	0.08
Sarcopenic obesity	103	0.77	0.30 – 1.96	0.59	1.23	0.45 – 3.35	0.68

Abbreviations: BMI = Body Mass Index, CI = Confidence Interval, FIGO = International federation of Gynecology and obstetrics, HR = Hazard ratio.
** Significant at p < 0.05 level*



Chapter 4

Systematic review on socioeconomic deprivation and survival in endometrial cancer

H. Donkers, R. Bekkers, L. Massuger, K. Galaal

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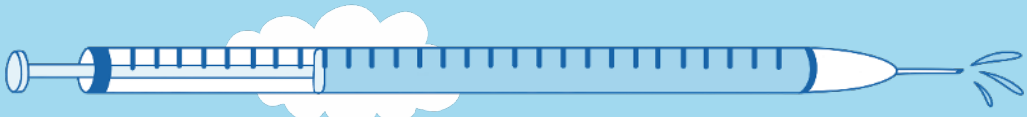

Abstract

Purpose
The primary objectives in this review were to (1) assess the association between socioeconomic deprivation and survival in endometrial cancer (EC) (2) investigate if there is an association between socioeconomic deprivation and peri-operative morbidity in EC.

Methods
We performed a systematic review using Medline (1946-2018), Embase (1980-2018), Cinhal (1981-2018) and the Cochrane Controlled Register of Trials to identify studies that reported on the association between socioeconomic deprivation and survival or peri-operative outcomes in EC. Included were adult women (age ≥ 18 years) diagnosed with primary EC. Two reviewers independently selected studies and assessed bias using the Newcastle-Ottawa assessment scale. Data extraction was completed using pre-determined forms, and summary tables of evidences from the included studies were created.

Results
Nine studies were included in this review with a total number of 369,900 patients. Eight studies investigated survival and socioeconomic deprivation, and the majority showed that socioeconomic deprivation is associated with poorer survival in EC. One study assessed the association between deprivation and peri-operative morbidity and found no difference in 30-day postoperative mortality.

Conclusions
Socioeconomic deprivation seems to be associated with worse survival in EC, even after adjusting for stage at diagnosis. However, the impact of important confounders such as BMI, smoking and comorbidities is unclear and should be assessed. The relationship between socioeconomic deprivation and peri-operative morbidity is unclear, and further research is needed to evaluate this aspect. A standardised measure for socioeconomic deprivation is needed in order to establish adequate comparison between studies.

Introduction

Endometrial cancer (EC) is the sixth most commonly occurring cancer in women, with over 380,000 estimated new cases worldwide and nearly 90,000 estimated deaths in 2018[1]. Both incidence and mortality rates have increased over the last decades, with obesity being one of the main risk factors[2]. The increase in obesity has multiple underlying factors including socioeconomical factors, with a strong association between obesity and lower socioeconomic status (SES) in EC and in the general population[3,4].

Socioeconomic status (SES) is a measure of an individual's economic and sociological standing and is based on income, education and occupation[5]. SES is considered to be an important predictor of health due to health inequalities[6]; however, the relationship between socioeconomic deprivation and cancer is complex and multifaceted. The incidence of various cancers including EC is higher in deprived groups[7]. Furthermore, death rates are examined extensively and are shown to be higher among the most deprived for most types of cancer[8]. However, the relationship between socioeconomic deprivation and survival in EC patients is not fully established.

Whilst Body Mass Index (BMI) is related to SES and obesity is associated with an increased risk of surgical morbidity in EC[9], the relationship between socioeconomic deprivation and peri-operative morbidity is unclear.

In this systematic review, our aim is to establish the relationship between socioeconomic deprivation and survival in EC. In addition, we aim to investigate the correlation between socioeconomic deprivation and peri-operative morbidity.

Objectives

- To evaluate the association between socioeconomic deprivation and survival in EC.
- To assess the correlation between socioeconomic deprivation and peri-operative morbidity in EC.

Methods

Study design

We conducted a systematic review of the literature to address the subject of socioeconomic deprivation and survival in EC.

Eligibility criteria

We have used the following definition by Peter Townsend, sociologist, of socioeconomic deprivation: a lack of social and economic benefits which are

considered to be basic necessities in a society[10]. We have included studies with individual, area-based or both types of measures of socioeconomic deprivation in this review.

The following criteria were used to exclude articles from further consideration: not in English, contained no original data, meeting abstract only (no full article for review) or article did not apply to any of the review questions. We furthermore excluded articles that used indirect measures of socioeconomic deprivation such as marriage or insurance status only.

Types of studies

We included all study designs evaluating the association between socioeconomic status and survival or peri-operative outcomes in EC as a primary outcome.

Types of participants

Adult women (age ≥ 18 years) diagnosed with primary EC.

Search strategy and selection criteria

This review was performed according to Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines, and in accordance with the principles outlined in the Cochrane Handbook[11]. We performed systematic searches in Medline (1946 until May 2018), Embase (1980 until May 2018) and Cinahl (1981 until May 2018) and the Cochrane Controlled Register of Trials. Search strategies were adapted accordingly to each database. The used search strategies are presented in **Appendix 1**. In addition, we searched grey literature including abstracts of scientific meetings as well as manually checking the reference lists of eligible studies to identify any additional studies to include in this review.

Types of outcome measures

Primary outcomes: Survival including overall survival (OS), cause-specific survival (CSS) and recurrence-free survival (RFS).

Secondary outcomes: Peri-operative morbidity in terms of peri-operative complications (intra-operative complications including nerve injury, bowel injury, bladder injury, ureter injury and vascular injury and post-operative complications including wound problems, fascia dehiscence, ileus, urinary tract infection, haemorrhage, pneumonia, pelvic abscess, haematoma, venous thromboembolism, sepsis, renal complications, cardiac complication and organ failure) and 30-day mortality.

Study selection

Two reviewers (HD and KG) independently assessed titles and abstracts of all

identified studies. Those studies that clearly did not meet the inclusion criteria were excluded. Potentially relevant studies were retrieved in full text and were further reviewed for eligibility by both reviewers.

Data extraction

Data extraction was completed by two of the authors (HD and KG) using pre-determined forms which included study author names, publication dates, study designs, sample sizes, measures of socioeconomic deprivation, results of univariate analyses testing for zero-order association between socioeconomic deprivation and survival or peri-operative outcomes and the results of the multivariate analyses testing for association between socioeconomic deprivation and survival or peri-operative outcomes adjusting for control variables. Differences were resolved by consensus.

Assessment of risk bias

The risk of bias included in studies was assessed by two authors (HD and KG) independently using the Newcastle-Ottawa Quality Assessment Form for Cohort Studies which includes selection, comparability and outcome[12]. Differences were resolved by consensus.

Data synthesis

We were unable to conduct a meta-analysis because of the heterogeneity in the included studies. However, we created summary tables of evidence from the included studies and then examined the relationship between various measures of socioeconomic deprivation and outcomes across studies.

Results

Study selection

The search strategy identified 127 references in Medline, 183 in Embase and 35 in Cinahl. Search results were merged, and duplicates were removed, resulting in 247 unique studies. After screening title and abstract, 16 articles were retrieved in full text and were further assessed for eligibility. Subsequently seven studies were considered eligible for this review, and a further search of the grey literature identified two articles, resulting in the inclusion of nine articles in this review (**Figure 1**).

Study characteristics

The characteristics of the nine studies included in this review are illustrated in **Table 1**. All studies combined resulted in a total of 369,900 EC patients, and all studies were of retrospective design. Eight studies included all FIGO stages and subtypes of EC, whereas one study only included FIGO stages I to II endometrioid endometrial carcinoma (EEC).

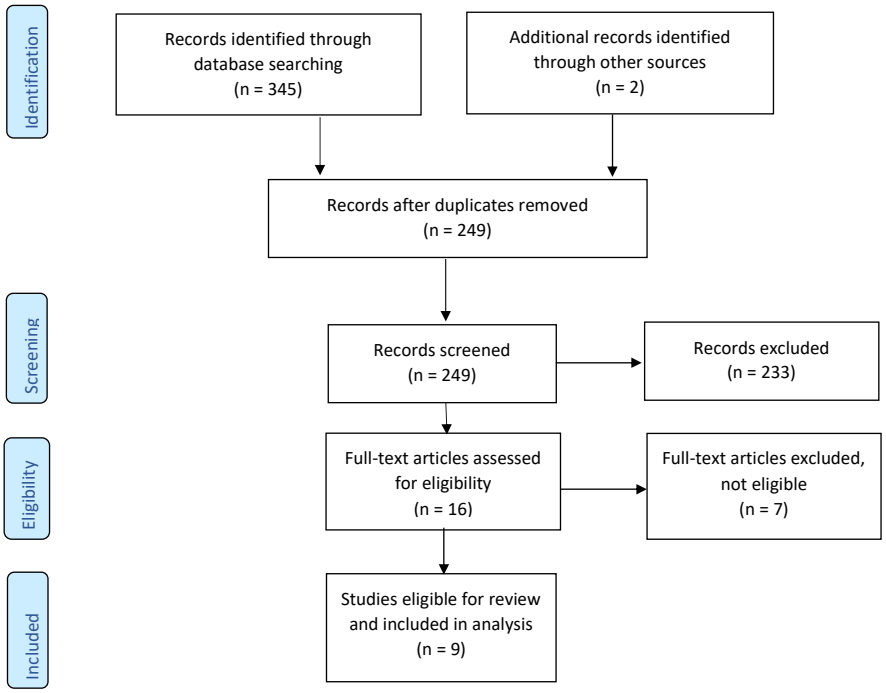

Figure 1. PRISMA flow diagram

Different measures of socioeconomic deprivation were used: income, level of education, unemployment, social class, housing tenure and insurance status. Furthermore, two studies used the Income Domain of the English Indices of Multiple Deprivation (IMD), which are published by the UK Department for Communities and Local Government and measure a spectrum of deprivation[13].

Risk of bias of included studies

We have assessed the risk of bias with the Newcastle-Ottawa scale. Two studies were of poor quality and seven studies were of good quality (**Figure 2**).

Synthesis of results

Socioeconomic deprivation and survival

Eight studies with 331,568 EC patients assessed survival and socioeconomic deprivation (**Table 1**). A study done by The National Cancer Intelligence Network (NCIN) used the income domain of IMD and showed a relationship between deprivation and mortality in England, with a higher mortality rate in the more income-deprived groups[14]. However, they did not adjust for any confounders.

Table 1. Characteristics of included studies.

Study	Population	No of patients	Measure of deprivation	Outcome measures	Conclusion
National Cancer Intelligence Network (NCIN) (2013)[14]	- EC patients - Median/mean age not reported for whole population - In United Kingdom (UK) - All types and stages	23,454	Income domain of English Indices of Multiple Deprivation	Age-standardised mortality rate (ASMR)	There is a relationship between deprivation and mortality in England, but not in other UK countries *Not adjusted for confounders*
Madison et al. (2004)[15]	- EC patients - Median age 65 years - In United States - All types and stages	3,656	Median household income	Survival time	A higher median household income was associated with a decreased risk for death *Adjusted for stage, age, treatment and histology*
Robbins et al. (2013)[16]	- EC patients - Median/mean age not reported for whole population - In United States - FIGO stages I to II EEC only	660	Median household income	Disease-specific survival (DSS), overall survival (OS), recurrence-free survival (RFS)	1. Worse OS and DSS in lower income groups 2. No difference in RFS *In multivariate analysis, not significant*
Fader et al. (2016)[17]	- EC patients - Mean age 61.9 years - In United States - All types and stages	228,511	Insurance status, median household income, education, facility location	Overall survival	Region of treatment facility and insurance status were associated with shorter overall survival but income and education were not *Adjusted for stage, age and treatment*
Cheung et al. (2013)[18]	- EC patients - Mean age 62.4 years - In United States - All types and stages	64,710	County level family income, county level % college graduate	Cause-specific 5-years survival (CSS)	Low income neighbourhoods decreased the CSS by 3% *In multivariate analysis, not significant*
Ueda et al. (2006)[19]	- EC and CC patients - Median/mean age not reported for whole population - In Japan - All types and stages	3,113	% of male unemployment, percentages of college or graduate school graduates within a municipality	5-years survival	Low SES (education and unemployment) was associated with poorer survival *Adjusted for stage, age and treatment*

table continues

Study	Population	No of patients	Measure of deprivation	Outcome measures	Conclusion
Jensen et al. (2008)[20]	- EC, CC and OC patients - Median/mean age not reported for whole population - In Denmark - Types and stages not reported	3,826	Level of education, disposable income, affiliation to the work market, social class, housing tenure and size of dwelling	One- and five-year relative survival	No strong association between socioeconomic variables and 1- and 5-year relative survival but tends to be better for women with higher education and more disposable income *Only adjusted for age*
Seidelin et al. (2016)[21]	- EC patients - Median age 65.5 years - In Denmark - All stages, types not reported	3,638	Level of education	Death from all causes	Short education is associated with a higher hazard ratio for death *Adjusted for stage, BMI, comorbidities and age*
Gildea et al. (2016)[22]	- EC patients - Median/mean age not reported for whole population - In England - All stages and types excluding sarcomas and malignant mixed Mullerian tumours	38,332	Income domain of English Indices of Multiple Deprivation	30-day post-operative mortality	No association between income deprivation and 30-day postoperative mortality

Abbreviations: EC = endometrial cancer, CC = cervical cancer, OC = ovarian cancer

Furthermore, three studies[15–17] used the median household income as measure of SES of which two studies (Madison et al. and Robbins et al.) found an association between mortality and deprivation, with a higher income being associated with a decreased risk of death[15,16]. However, in multivariate analysis Robbins et al. did not identify SES as a significant predictor of patient outcome, while in the study done by Madison et al. the significance remains after adjusting for stage. The third study done by Fader et al. found no difference in overall- or recurrence-free survival within the different income groups or educational levels, even after adjusting for stage[17].

	Selection	Comparability	Outcome/exposure
NCIN, 2013	* * *	-	* * *
Madison, 2004	* * *	*	* * *
Robbins, 2013	* * *	*	* * *
Fader, 2016	* * *	*	* * *
Cheung, 2013	* * *	-	* * *
Ueda, 2006	* * *	*	* * *
Jensen, 2008	* * *	*	* * *

Figure 2. Risk of bias *(Newcastle-Ottawa Scale)*

The study done by Cheung et al. looked at county-level family income and found a decreased survival in patients living in low-income neighbourhood[18], which did not remain significant in multivariate analysis, while a study done by Ueda et al. used municipality-based SES and found poorer 5-year survival in the low unemployment and education municipalities, even after adjusting for stage[19]. The study done by Jensen et al. used six different socioeconomic indicators and found no strong association between socioeconomic variables and survival, but survival tended to be better for women with higher education and more disposable income, however they did not correct for confounders[20]. Lastly, a study done by Seidelin et al. showed education to be associated with higher hazard ratio (HR) for death, even after adjusting for confounders such as stage at diagnosis, BMI and comorbidities[21].

Socioeconomic deprivation and peri-operative morbidity

A study done by Gildea et al. used the income domain of the English IMD to assess the relationship between postoperative mortality and deprivation in EC, and found no association between income deprivation and 30-day postoperative mortality[22]. No other articles were found, which investigated the association between deprivation and peri-operative morbidity in EC.

Discussion

This review summarises the current literature about the association between socioeconomic deprivation and survival in EC. The results of this systematic review suggest a worse survival for more socioeconomically deprived patients, with six studies showing low SES being associated with worse survival in univariate analysis, and three studies confirming poor outcome in multivariate analysis. However, two studies did not show an association.

Previous research has looked at possible explanations for the differences in cancer survival within different groups of deprivation, with stage at diagnosis being one of the most important factors[23]. In cervical-, breast- and colorectal cancer, these survival differences are related to the differences in participation rate in cancer screening programmes, in which women with lower SES and women living in urban areas are less likely to participate[24]. For EC there is no routine screening; however, since patients present early with bleeding problems, it is usually diagnosed at an early stage, which leads to high survival rates[14]. This suggests that SES impacts survival in EC through other factors which may include BMI, age, smoking and comorbidities.

For EC, factors that are associated with advanced stage at diagnosis include high-grade lesions, serous histologic subtype, older age and low SES[21,25,26]. Patients with higher socioeconomic position might be more aware of symptoms and present quicker to a general practitioner or medical specialist, while low SES patients tend to ignore early symptoms of disease such as postmenopausal bleeding[21]. This could partially explain the differences in survival, however most studies included in this review with the exception of two (Jensen et al. and NCIN) corrected for stage at diagnosis in their analyses. Therefore, it seems that regardless of stage at diagnosis, socioeconomic deprivation affects survival in EC.

Other important factors in cancer survival in general and in EC include BMI, with normal-weight women having better survival than obese patients[23,27]. One of the mechanisms that has been suggested to explain the differences in survival is the fact that obesity is associated with an increased risk of surgical morbidity[9]; however some studies have shown that it is not an independent predictor but likely related to other comorbid conditions[28]. Since there is a strong relationship between obesity and socioeconomic deprivation in EC[4], this could potentially be an important factor affecting survival in deprived patients; however the articles included in this review, with the exception of Seidelin et al., did not include or correct for BMI.

A third factor in cancer survival is comorbidity, previous research has shown a decreased survival for EC patients with multiple comorbidities[29]. Different reasons

are reported such as delayed diagnosis, higher rate of postoperative complications, a reduction of the possibility of surgery and a lower tolerance of oncological treatment[30]. Since the prevalence of comorbidity tend to be higher among EC patients with higher levels of deprivation[31], this could also affect survival. Of the articles included in this review, only Seidelin et al. corrected for comorbidity and found no difference in the odds ratio for death.

Furthermore, age at diagnosis is an important factor in EC survival[32]. Elderly patients often have more aggressive histology and are less likely to receive surgical treatment or adjuvant therapy leading to under treatment[33]. Furthermore, an article by Poupon et al. showed 3-year OS rates to be lower than cancer specific survival rate, indicating that death in elderly is often a combination of death due to cancer as well as to causes other than cancer[33]. All studies included in this review have adjusted for age at diagnosis with the exemption of Cheung et al[18].

Another element in survival in EC is treatment received by patients[34]. This is often influenced by patient characteristics such as age, comorbidities and patient preference. Furthermore, the type of treatment centre (cancer centre or smaller hospital) also influences the type of treatment offered to patients and influences survival in EC[35,36]. Patients with low SES are less likely to afford travel costs to cancer centres, especially if they reside in rural counties. Only half of the studies included in this review have taken treatment into account.

Lastly, smoking status is an important aspect in survival in EC, in which smokers show worse survival compared to non-smokers[37], although some of the overall survival differences may be more attributable to associated co-morbid conditions in smokers. None of the studies in this review have corrected for smoking status in their analysis.

BMI, comorbidities and smoking not only affect survival, but are also risk factors for peri-operative morbidity in EC[9,38,39]. Because of the correlation of SES with BMI, comorbidities and smoking status, we have tried to investigate if there is a relationship between socioeconomic deprivation and peri-operative morbidity, however the current literature is scarce and only one study was identified which did not show an association between income deprivation and 30-day postoperative mortality[22]. Further research is needed to establish any relationship between socioeconomic deprivation and peri-operative morbidity including 30-day mortality in EC.

The studies in this review have used different measures of mortality (age-standardised mortality rate, survival time, disease-specific survival, overall survival, 1- and 5-year survival etc.), which is an important issue when comparing

results. EC has a relatively high survival rate; however, the 1-year survival will be very different to mortality rates and may reflect the individual's underlying comorbidities which may lead to earlier demise. Therefore, it is difficult to compare all different measures of mortality.

Despite increasing recognition of the impact of socioeconomic deprivation on survival of EC, questions about the strength of its impact and relationship with other prognostic factors remain. To address these questions, more studies are needed which measure socioeconomic deprivation with a standardised measure and also correct for other prognostic variables including BMI, comorbidities and smoking status. From this knowledge, interventions to improve survival in lower SES patients can then be introduced.

Overall completeness and applicability of evidence

The majority of women were diagnosed with stage I disease, consistent with reported incidence rates[40]. Literature was scarce about the correlation between socioeconomic deprivation and peri-operative morbidity and we only found one study that evaluated peri-operative outcomes and SES in EC.

Quality of evidence

The studies included were of a high degree of heterogeneity in study design and evaluated a variety of socioeconomic status measures, which lacked in uniformity. Different measures were used, each capturing a distinct aspect of SES, which may be correlated with other measures, but are not interchangeable[41]. Furthermore, individual SES measures such as income and occupation differ from environmental SES measures, which are area-based, and these two measures often do not correlate well[42]. These different measures can all impact results. Area-based measures may not accurately represent a patient's socioeconomic deprivation status, since not all people living in a poor area are poor themselves. Furthermore, there is a large variety in definitions of socioeconomic deprivation: the definition of a deprived person living in the United States might be different from a deprived person living in Japan. In addition, in some studies only one indicator of SES was used, while others used multiple measures. Furthermore, most studies have not adjusted for important confounders. Therefore, it is difficult to draw hard conclusions about the strength of the evidence.

Potential biases in review process

A comprehensive search of the literature with aid of librarian was performed by two reviewers independently, including a search of the grey literature. Reviewers assessed potentially eligible articles independently and discussed the differences found.

Comparison with existing literature

A previous review done by Kogevinas et al. about socioeconomic differences in cancer survival included six studies about EC and showed survival was poorest in low socioeconomic groups in five studies; in three of those studies differences were statistically significant. However, the reverse pattern was seen in one study[8]. The association of inequality in survival is supported by several studies that have assessed the association between SES and cancer survival in general and that also included EC patients[43,44,53,45–52], even though several other studies did not find an association between deprivation and mortality[54–58].

Conclusion

Socioeconomic deprivation seems to be associated with poorer survival in EC, regardless of their stage at diagnosis. However, important confounders such as BMI, comorbidities and smoking status should be taken into account. The relationship between peri-operative morbidity and socioeconomic deprivation in EC is not clear.

Implications for clinical practice

- Socioeconomic deprivation status should be included in the initial evaluation of new patients diagnosed with endometrial cancer
- Early and non-invasive diagnostic testing is needed in the community to improve access to health care in deprived groups
- Early referral to cancer support teams is recommended

Implications for research

- Further research should be directed to establish any relationship between socioeconomic deprivation and peri-operative morbidity in endometrial cancer
- Furthermore, a standardised measure for socioeconomic deprivation is needed in order to establish adequate comparison between studies
- Further studies should adjust for important confounders such as BMI, comorbidities and smoking to assess the true extent of socioeconomic deprivation on survival in endometrial cancer

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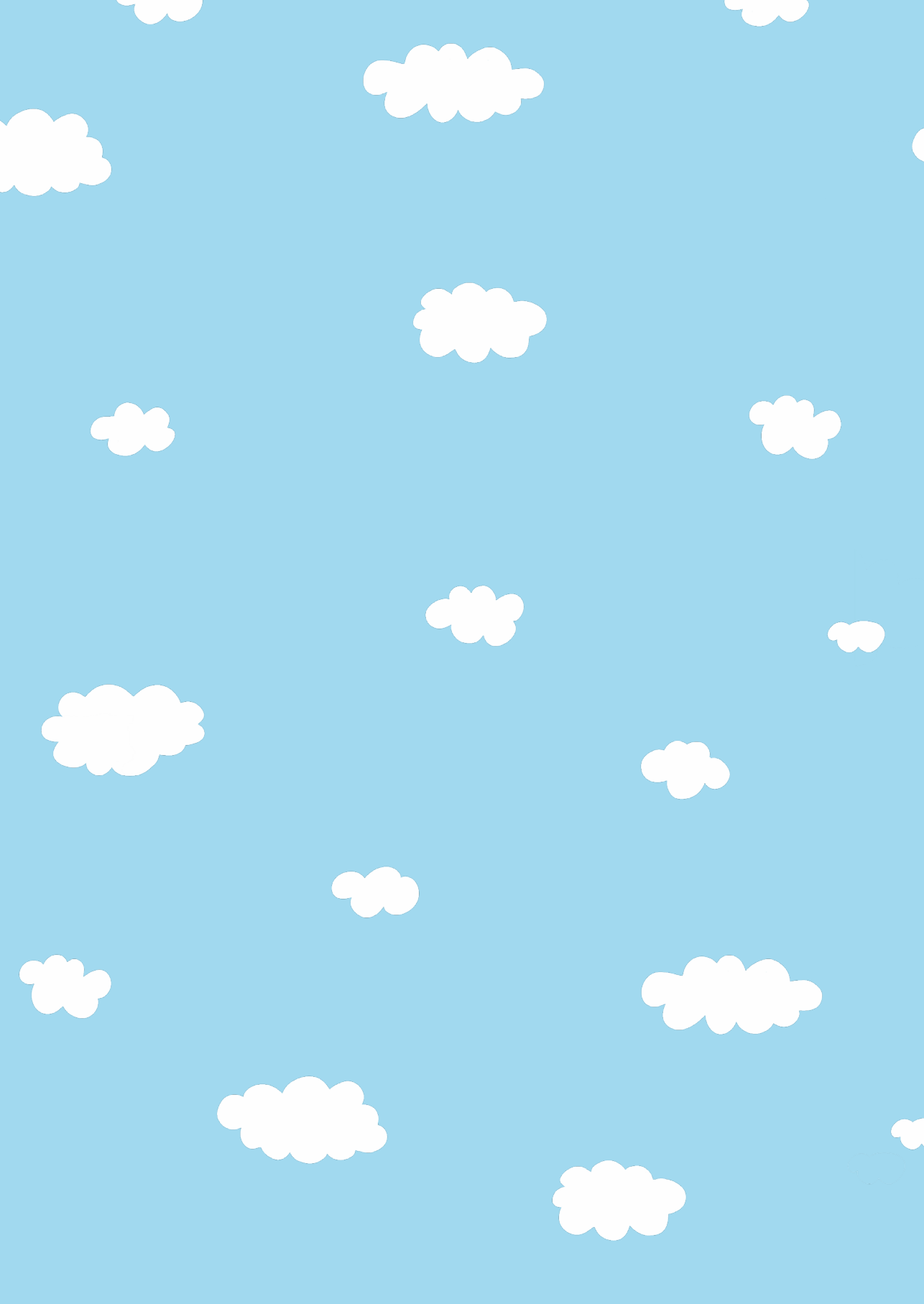
Supplemental material

Appendix 1. Search strategy

1. endometr* adj2(neoplas* OR carcinom* OR cancer* or tumour* OR tumour)
2. exp endometrial neoplasms/
3. 1 OR 2
4. deprivation
5. deprived
6. socioecon*
7. poverty
8. ''social class''
9. 4 OR 5 OR 6 OR 7 OR 8
10. survival
11. survived
12. survivors
13. outcome
14. outcomes
15. complication
16. complications
17. morbidity
18. mortality
19. death
20. died
21. 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20
22. 3 AND 9 AND 21



Chapter 5

Socioeconomic deprivation and survival in endometrial cancer: The effect of BMI

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Gynecologic Oncology.
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Abstract

Objectives

- To determine the association between survival and socioeconomic deprivation in endometrial cancer (EC).
- To evaluate and quantify the effect of socioeconomic deprivation on peri-operative morbidity and mortality in EC.

Methods

This is a retrospective study of surgically managed EC patients in the Royal Cornwall Hospital Truro between January 2006 and August 2017. Patient characteristics, overall survival, recurrence-free survival and intra- and postoperative outcomes were evaluated across socioeconomic deprivation groups in which socioeconomic deprivation was measured with the English Indices of Multiple Deprivation (IMD).

Results

In total, we identified 831 women, of which 690 were included. The median age was 66 years with a median BMI of 31 kg/m^2 and the majority of tumours were endometrioid endometrial carcinomas (EEC, 80%). In EEC, better survival was seen in the least deprived patients, however this was not significant in multivariate analysis and only age, stage and BMI remained significantly associated. For non-endometroid endometrial carcinoma (NEEC), no association between survival and socioeconomic deprivation was found and only stage was significant. However, more affluent patients had significantly higher recurrence rates. In addition, we did not find evidence of an association between intra- or post-operative complication rates and socioeconomic deprivation.

Conclusion

Socioeconomic deprivation is associated with survival in EC, however after adjusting for confounders this association does not remain. Only age, stage and BMI are independent prognostic factors for survival. In addition, there is no evidence of association between socioeconomic deprivation and peri-operative outcomes in EC.

Introduction

Endometrial cancer (EC) is the most common gynaecological malignancy in the United Kingdom (UK), with over 9,000 women being diagnosed annually[1]. The most important risk factor for the development of EC is obesity[2], which is associated with low socioeconomic status (SES)[3,4]. SES is the social standing or class of an individual or group and is measured as a combination of income, education and occupation[5]. Socioeconomic deprivation is considered to be an important predictor of health and survival with major inequalities among different levels of deprivation[6].

For most types of cancer, socioeconomic deprivation is associated with survival, with more deprived patients showing poorer survival[7]. A recent systematic review showed that socioeconomic deprivation was associated with poorer survival in EC, even after adjusting for stage at diagnosis[8]. However, not only stage at diagnosis is associated with worse survival, but grade of disease, obesity and comorbidities are also related to survival[9,10]. The exact interaction between these factors, socioeconomic deprivation and survival in EC are unclear.

More deprived patients tend to have higher Body Mass Index (BMI) and more comorbidities[11]. In addition, obesity and multiple comorbidities are associated with an increased risk of surgical morbidity in EC[12]. However, the relationship between socioeconomic deprivation and peri-operative morbidity in EC is unclear. The aim of this study was to investigate the association between survival and socioeconomic deprivation in EC in more detail while taking into account confounding factors such as BMI and comorbidities. In addition, we aim to evaluate and quantify the effect of socioeconomic deprivation on peri-operative morbidity and mortality in patients undergoing surgery for EC.

Materials and methods

Study design and population

This is a retrospective cohort study in the Royal Cornwall Hospital Trust (RCHT) in Truro, UK, on women undergoing surgical treatment for EC. Women with histologically proven primary EC receiving surgical treatment at the RCHT between January 2006 and August 2017 were identified from the cancer registry of the South West Cancer Intelligence Service. Exclusion criteria were: less than 18 years of age, incomplete follow-up data and unknown address. Full ethical approval was obtained through Health Research Authority (HRA), No: 236426 and the study had full Trust approval.

Data collection

Patient characteristics including date of diagnosis, age at diagnosis, stage, grade, histology, treatment modality, intra- and postoperative complications and other clinical characteristics were collected from the medical records. The patients postal code was used to identify the geographical location of residence, so that it could be assigned a deprivation risk using the English Indices of Multiple Deprivation (IMD) 2015[13].

IMD are published by the UK Department for Communities and Local Government and measure a spectrum of deprivation, combining 38 indicators that are grouped into seven domains which are differently weighted (income deprivation 22.5%, employment deprivation 22.5%, health deprivation and disability 13.5%, education skills and training deprivation 13.5%, barriers to housing and services 9.3%, living environment 9.3% and crime 9.3%). IMD gives an overall measure of deprivation experienced by people living in a certain area for every Lower Layer Super Output Area (LSOA) in England. There are 32,844 LSOAs in England, where 1 is the most deprived LSOA and 32,844 is the least deprived.

Outcome measures

Primary outcome: overall survival (OS) defined as the length of time from date of diagnosis that patients are still alive and recurrence-free survival (RFS) defined as the length of time after primary treatment ends that patients survive without any signs or symptoms of cancer.

Secondary outcomes: surgical morbidity in terms of intra-operative complications (including bowel injury, bladder injury, ureter injury, vascular injury), post-operative complications (including wound problems, fascia dehiscence, ileus, antibiotics usage, urinary tract infection, haemorrhage, pneumonia, pelvic abscess, haematoma, venous thromboembolism, sepsis, renal problems, cardiac problems, organ failure, relaparotomy, readmission) and other surgical outcomes such as hospital stay, blood loss, transfusion and 30- and 90-day mortality.

Statistical analysis

For analysis purposes, deprivation data was grouped into 3 groups, with 1 being the most deprived and 3 being the least deprived. BMI was recorded and categorised according to national guidelines: underweight (≤ 18.5 kg/m^2), normal range (18.5 – 24.9 kg/m^2), overweight (25 – 29.9 kg/m^2) and obese (≥ 30 kg/m^2)[14].

For normal distributed data continuous outcomes were presented as means or medians and standard deviations, categorical data were presented as frequencies and proportions. Non-parametric continuous data was compared using the Kruskal-Wallis test or Mann-Whitney U test, and Pearson Chi-square test or Fisher's Exact

test for categorical data. Survival data were estimated using Kaplan-Meier curves and Cox Regression models were used while controlling for possible confounders. The statistical significance of differences in outcome between the groups was assessed with the log-rank test. P-values less than 0.05 were considered significant for all tests. Data were analysed with IBM SPSS statistics version 24.0.

Results

A total of 829 women were diagnosed with EC at the RCHT between January 2006 and August 2017, of which 722 had undergone surgical treatment. Excluded were 19 women who received treatment elsewhere, 14 women with incomplete data on their perioperative course and one woman with an unknown postal code. Consequently, our study population consisted of 688 patients (**Figure 1**).

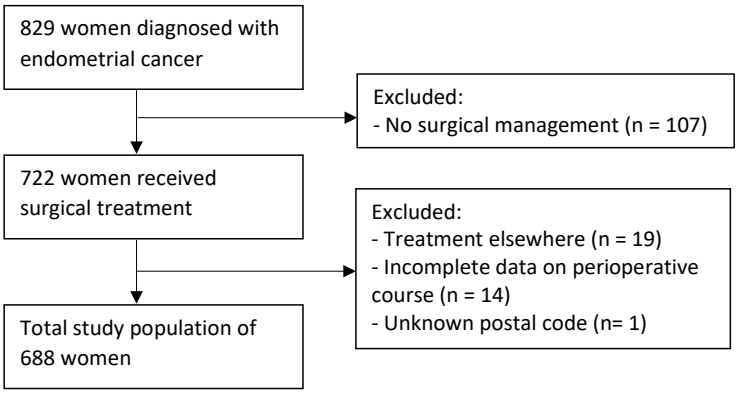

Figure 1. Flow chart of recruitment

Baseline characteristics

The median age of included patients was 66 years (SD 10.6) with a median BMI of 31 kg/m^2 (SD 7.2) (**Table 1**). Majority of tumours were endometrioid tumours (8%), followed by serous (6%) and carcinosarcomas (5%). Most women presented with FIGO stage I disease (82%), followed by stage III (7%), stage II (7%) and stage IV (3%). The majority of women had two or more comorbidities (53%). Furthermore, 118 women (17%) died of which 67 (57%) due to EC and 42% due to other causes such as cardiac or respiratory diseases. In addition, 76 (11%) had recurrent disease. The median RFS time was 48.4 months.

Table 1. Baseline characteristics.

Characteristics	Value
No. of patients	688
Age (years), median	66
BMI (kg/m^2), median	31
BMI groups (%)	
< 25	117 (17)
25 – 30	178 (26)
> 30	370 (54)
Unknown	23 (3)
Ethnicity (%)	
Caucasian	679 (99)
Any other ethnic group	6 (1)
Unknown	3 (0)
Smoking (%)	
Yes	49 (7)
No	562 (82)
Ex-smoker	14 (2)
Unknown	63 (9)
Parity (%)	
0	114 (17)
1	110 (16)
≥2	388 (56)
Unknown	76 (11)
Comorbidities (%)	
None	156 (23)
One	162 (24)
Two or more	365 (53)
Unknown	5 (1)
Histology (%)	
Endometrioid	552 (80)
Serous	40 (6)
Carcinosarcoma	33 (5)
Mixed tumours	17 (3)
Clear cell	13 (2)
Mucinous	5 (1)
Other	21 (3)
Unknown	7 (1)
FIGO stage (%)	
I	564 (82)
II	47 (7)
III	51 (7)
IV	20 (3)
Unknown	6 (1)
Grade (%)	
1	257 (37)
2	243 (35)
3	151 (22)
Unknown	37 (5)
Treatment modality (%)	
Laparoscopic hysterectomy +/- BSO	355 (52)
Laparotomy hysterectomy +/- BSO	318 (46)
Other type surgery	10 (2)

table continues

Characteristics	Value
Unknown	5 (1)
Adjuvant treatment (%)	
Neoadjuvant	13 (2)
Adjuvant	195 (28)
None	464 (67)
Unknown	16 (2)
Type (neo)adjuvant treatment (%)	
Chemotherapy	89 (43)
Radiotherapy	166 (80)
Hormonal treatment	33 (16)
Recurrence (%)	
Yes	76 (11)
No	596 (87)
Progression despite treatment	11 (2)
Unknown	5 (1)
Death (%)	
Yes	118 (17)
No	570 (83)
Cause of death (%)	
Endometrial cancer	67 (56)
Other	51 (43)
Deprivation groups (%)	
Most deprived	255 (37)
Middle deprived	394 (57)
Least deprived	37 (5)
Unknown	2 (0)

Abbreviations: BMI = Body Mass Index, FIGO = International Federation of Gynecology and Obstetrics

Socioeconomic deprivation and survival

Initially all types of EC combined were analysed. The worst OS was seen for the middle-deprived group, with similar OS for the least and most deprived groups (p = 0.007, **Figure 2**).

The mean survival time was 123 months (95% CI 118 - 129) for the most deprived patients, 111 months (95% CI 106 – 117) for the middle-deprived group and 118 (95% CI 101 – 133) for the least deprived group of patients. However, when performing a Cox Regression analysis in which we corrected for age at diagnosis, BMI, comorbidities, smoking status, histology, stage, grade and type of surgery, deprivation was not significantly associated with survival (p = 0.237). Only stage (p < 0.001, Risk Ratio (RR) 2.197), grade (p = 0.044, RR 1.381) and age at diagnosis (p < 0.001, RR 1.075) were significantly associated with survival.

For further analyses we subdivided our patients into two categories; endometrioid endometrial carcinoma (EEC) versus non-endometrioid endometrial carcinoma (NEEC). In EEC, better OS for the least deprived patients was seen (p = 0.034, **Figure 3**).

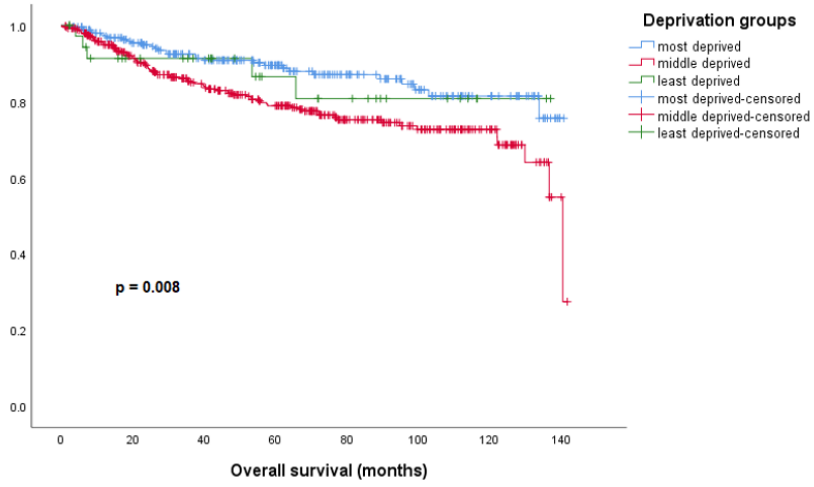

Figure 2. Kaplan-Meier plots showing overall survival for different deprivation groups in all endometrial cancer patients

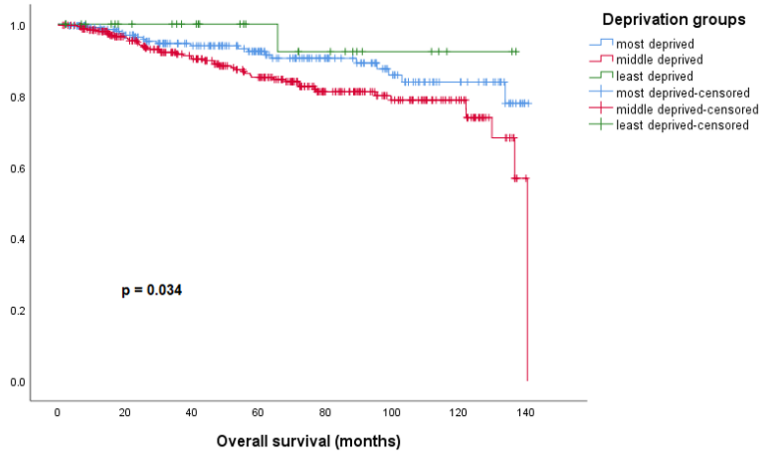

Figure 3. Kaplan-Meier plots showing overall survival for different deprivation groups in EEC

The mean survival time was 127 months (95% CI 122 – 133) for the most deprived patients, 118 months (95% CI 113 – 124) for the middle-deprived group and 132 (95% CI 119 – 126) for the least deprived patients. When performing a Cox

Regression analysis, only age at diagnosis (p < 0.001. RR 1.091), stage (p < 0.001, RR 2.652) and BMI (P = 0.043, RR 1.041) remained significantly associated with survival.

For NEEC, least deprived patients seemed to show the poorest survival, however this was not statistically significant and numbers were small (p = 0.064, **Figure 4**).

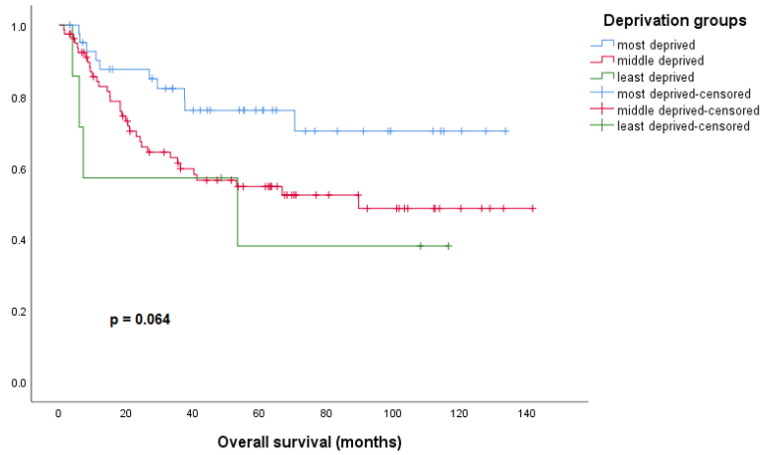

Figure 4. Kaplan-Meier plots showing overall survival for different deprivation groups in NEEC

For NEEC, multivariate Cox Regression analysis showed only stage (p = 0.003, RR 1.796) to be associated with survival.

The overall one-year and five-year survival was 95% and 83% respectively. For EEC the one-year and five-year survival was 98% and 88% respectively compared to 82% and 61% for NEEC.

Socioeconomic deprivation and recurrence

The total recurrence rate was 11% (n = 76) with median time until recurrence of 15.9 months (SD 22). In total, 19 patients (8%) in the most deprived group, 49 (13%) in the middle-deprived group and 8 (22%) in the least deprived group had a recurrence, which is statistically significant (p = 0.015). When analysed for the subgroups, EEC did not show a significant association between socioeconomic deprivation and recurrence rates (p = 0.590), however NEEC did show a significant (p = 0.017) higher recurrence rate for patients with a better socioeconomic position.

Socioeconomic deprivation and intra-operative complications

In total, 45 patients developed intra-operative complications, but there was no significant difference (p = 0.844) in incidence rates among different deprivation groups. No difference was found for the different types of intra-operative injuries, including bowel, bladder, ureter and vascular injuries (**Table 2**).

Table 2. Surgical complications and outcomes according to deprivation groups.

Variables	Most deprived N = 255	Middle deprived N = 394	Least deprived N = 37	Analysis p-value
Intra-operative complications				
Yes	18 (7)	24 (6)	3 (8)	0.844
No	237 (93)	365 (92)	34 (92)	
Individual complications				
Bowel injury	2 (1)	1 (0)	1 (3)	0.150
Bladder injury	1 (0)	0 (0)	0 (0)	0.428
Ureter injury	1 (0)	0 (0)	0 (0)	0.428
Vascular injury	3 (1)	10 (3)	1 (3)	0.472
Other	1 (0)	2 (1)	0 (0)	0.897
Post-operative complications				
Yes	54 (21)	83 (21)	8 (22)	0.998
No	201 (79)	308 (78)	29 (78)	
Individual complications				
Wound	16 (6)	21 (5)	3 (8)	0.745
Fascia dehiscence	3 (1)	4 (1)	1 (3)	0.663
Ileus	5 (2)	3 (1)	0 (0)	0.307
Antibiotics use	42 (17)	55 (14)	5 (14)	0.683
UTI	10 (4)	9 (2)	1 (3)	0.489
Haemorrhage	0 (0)	2 (1)	2 (5)	**0.000***
Pneumonia	6 (2)	7 (2)	0 (0)	0.601
Pelvic abscess	4 (2)	0 (0)	0 (0)	**0.035***
Haematoma	4 (2)	3 (1)	0 (0)	0.502
VTE	2 (1)	1 (0)	0 (0)	0.561
Sepsis	0 (0)	2 (1)	0 (0)	0.473
Renal	3 (1)	3 (1)	0 (0)	0.725
Cardiac	5 (2)	10 (3)	1 (3)	0.873
Organ failure	1 (0)	4 (1)	0 (0)	0.568
Relaparotomy	4 (2)	5 (1)	3 (8)	**0.010***
Readmission	17 (7)	23 (6)	6 (16)	0.059
30-day mortality	0 (0)	3 (1)	0 (0)	0.325
90-day mortality	1 (0)	6 (2)	0 (0)	0.304
EBL, mean (SD)	196 (190)	173 (194)	190 (366)	0.545
Transfusion	2 (1)	10 (3)	1 (3)	0.256
Hospital stay, mean (SD)	4.9 (4)	4.6 (2.9)	4.2 (2.2)	0.732
ICU requirement	15 (6)	17 (4)	1 (3)	0.556

Abbreviations: EBL = estimated blood loss, CU = intensive care unit, SD = standard deviation UTI = urinary tract infection, VTE =venous thromboembolism

Data are numbers of patients (%) unless stated otherwise. Numbers do not add up because of missing values

**: p < 0.05*

Socioeconomic deprivation and post-operative complications

In total, 145 patients developed post-operative complications, but there was no significant difference (p = 0.998) in incidence rates among different deprivation groups (**Table 2**). There was a significant difference in the incidence of haemorrhage (p < 0.001), pelvic abscess (p = 0.034) and relaparotomy (p = 0.010) among the different deprivation groups.

Crude 30-day postoperative mortality rates were low, with only 3 deaths, accounting for 0.45% of women treated surgically. 90-day postoperative mortality rates were similarly low, with 7 deaths reported, accounting for 1.01% of women treated surgically.

Discussion

The aim of this study was to determine the relationship between socioeconomic deprivation and survival in EC in more detail. We recently published a systematic review about socioeconomic deprivation and survival in EC, showing deprivation seems to be associated with worse survival, however the impact of important confounders such as BMI, smoking and comorbidities were unclear and therefore should be assessed[8]. In this study we found an association between socioeconomic deprivation and overall survival in EC, with better survival for the least deprived patients in EEC. However, this association was not found after adjusting for confounders. Furthermore, socioeconomic deprivation was not associated with survival in NEEC.

To our knowledge, this is the first study to assess survival and EC while looking at two different groups; EEC (associated with unopposed oestrogen stimulation and obesity) and NEEC (developing in the presence of endometrial atrophy in mostly elderly patients, including serous tumours, carcinosarcomas and clear cell tumours)[15]. For EEC, age at diagnosis, stage and BMI were associated with OS and for NEEC, only stage was associated with survival in multivariate analysis. Therefore, to improve survival rates in EC, it is not only important to focus on early detection and diagnosis but also on obesity, mainly in endometroid tumours, which is the vast majority (80%) of EC.

Furthermore, this is the first study assessing the association between recurrence and socioeconomic deprivation in EC. We showed recurrences rates to be significantly higher in less deprived patients, which is an unexpected finding. We would expect an inverse association, since patients with lower SES more often ignore symptoms of disease or recurrence and are more reluctant in approaching the healthcare system outside of scheduled appointments when they experience

symptoms[16]. Furthermore, more deprived patients tend to have more comorbidities and studies have shown more comorbidities to be associated with a higher risk of recurrence in EC[17]. Therefore, further research is needed to fully understand the link between recurrences and socioeconomic deprivation.

Finally, we investigated if there is a correlation between socioeconomic status and peri-operative outcomes in EC. This study showed a total intra-operative complication rate of 6.6% and a postoperative complication rate of 21.2%, which is in line with previous research[18]. However, we did not show a difference in intra-operative or postoperative complication rate in EC for different levels of deprivation. Only a small number of patients were identified with complications in all groups, which makes it difficult to draw any hard conclusions.

Socioeconomic deprivation and 30-day postoperative mortality has been investigated in colorectal cancer, in which deprivation was associated with poorer 30-day postoperative mortality even after adjusting for stage, comorbidity and urgency of surgical resection[19]. In our study, 30-day postoperative mortality for women with EC was low at 0.4%, which is in line with previous research[20], with no association between socioeconomic deprivation and 30-day postoperative mortality. In addition, 90-day postoperative mortality was low as well at 1.0% and again there was no association between socioeconomic deprivation and 90-day postoperative mortality. Our findings are in line with a study by Gildea et al. which also shows no differences in postoperative mortality rates in EC for different levels of overall deprivation[20].

We did not find an increased length of hospital stay after surgery in more deprived patients. Furthermore, more deprived patients did not require more intensive care unit (ICU) stay than less deprived patients. This is in contrast to a study done by Smith et al. in colorectal cancer patients, where social deprivation was found to be an independent risk factor of postoperative length of stay, despite the effect being relatively small[21]. This difference might be explained by the fact that postoperative length of stay is shorter for EC (4-5 days) compared to colorectal cancer patients (11-12 days), making it more difficult to establish a difference between different groups.

Strengths and limitations

Strengths of this study include the study size, the inclusion of all histological subtypes and stages and the subdivision of EC in two different subtypes. Our study has limitations due to its retrospective design, including non-randomization and completeness of previously recorded data. In addition, this is a single institution study, and therefore population level variations in quality of care due to low quality providers and institutions in poor neighbourhoods are not accounted for.

Furthermore, the Indices of Multiple Deprivation have limitations. They are based on geographical areas rather than individual (not all deprived people live in deprived areas and vice versa) and they are relative, rather than absolute measures of deprivation. However, since they cover a wide range of socioeconomic indicators, we believe it to be an accurate measurement for socioeconomic deprivation which has been used extensively in previous research[22–24].

Conclusion

Socioeconomic deprivation is associated with survival in EC; however, this does not remain after correcting for confounders. Only age at diagnosis, stage and BMI are independent prognostic factors for survival. Furthermore, recurrence rates are higher in more affluent patients. In addition, this study shows no association between deprivation and peri-operative morbidity and mortality in EC.

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Chapter 6

Abnormal pre-operative haematological parameters in endometrial cancer; reflecting tumour aggressiveness or poor health status?

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Submitted

Abstract

Background
In endometrial cancer (EC), haematological parameters such as haemoglobin level, platelet- and leucocyte counts have shown to be of prognostic relevance. Yet, it remains unclear whether these three parameters reflect tumour aggressiveness or merely indicate a poorer health status. We hypothesise that anaemia and thrombocytosis reflect tumour aggressiveness, whereas leucocytosis may also reflects a poorer health status including obesity and comorbidity, with impacting survival.

Objectives
To define the relationship of pre-operative anaemia, thrombocytosis and leucocytosis with clinicopathological characteristics in EC patients. Furthermore, to explore the prognostic relevance in terms of survival of these haematological parameters in EC patients, taking into consideration their comorbidities.

Study design
A retrospective multicentre cohort study of women diagnosed with endometrial cancer was performed in 10 hospitals. Pre-operative haematological parameters including haemoglobin level, platelet- and leucocyte counts were collected. Defined cut-offs were; anaemia: haemoglobin <7.45mmol/L (<12 g/Dl), thrombocytosis: platelets >400x10^9 platelets/L, and leucocytosis: leucocytes >10x10^9/L. Correlations-, Kaplan Meier- and Cox-regression analysis were performed.

Results
A total of 914 women were included. Anaemia was present in 103 (11.3%), thrombocytosis in 79 (8.6%) and leucocytosis in 114 (12.7%) women. Anaemia was correlated with thrombocytosis, and both were associated with advanced-stage disease (Federation International of Gynecology and Obstetrics (FIGO) III-IV) (P < 0.001). Leucocytosis was significantly associated with diabetes mellitus (DM), Body Mass Index (BMI) >35 kg/m^2 and advanced-stage disease (P < 0.001). Patients with leucocytosis and DM or high BMI did not show worse outcomes compared to patients without DM or high BMI. Anaemia was significantly associated with decreased overall- and disease-specific survival, whereas thrombocytosis was associated with significant decreased disease-specific survival. Furthermore, age ≥ 65 years, anaemia, grade 3 tumour histology and advanced-stage disease (FIGO III-IV) were independently associated with reduced overall- and disease-specific survival.

Conclusion
Pre-operative anaemia, thrombocytosis and leucocytosis are related to tumour aggressiveness in EC. Anaemia is the most important prognostic factor, whereas

leucocytosis is more related to obesity and comorbidity and reflects a poorer health status, without impacting survival. Abnormal pre-operative haematological parameters can be used to identify patients at risk for advanced-stage disease and/or need for additional surgical or adjuvant treatment.

Introduction

Endometrial cancer (EC) is the most common gynaecologic malignancy in industrialized countries with incidence rates rising due to aging population and increased rates of obesity[1]. Most patients are diagnosed at an early-stage and therefore have a favourable prognosis, whereas patients with advanced-stage disease have a high risk of recurrence and poor outcome[1,2].

Pre-operative risk stratification is important to identify patients with increased disease-related risk of mortality. Currently, primary surgical and subsequent adjuvant treatment strategies in EC are mostly based on pre-operative tumour type and grade of an endometrial biopsy, and final histopathological FIGO (Federation International of Gynecology and Obstetrics) stage[3]. Available clinical biomarkers such as haematological parameters might contribute to improved risk stratification by identifying patients with advanced-stage disease implicating aggressive tumour behaviour[4].

Endometrial carcinogenesis is characterized by chronic inflammation with elevated pro-inflammatory cytokines and acute phase proteins[5]. Overexpression of inflammatory cytokines could cause shortened survival of red blood cells, suppression of erythroid progenitor cells, impaired iron utilisation, and inadequate erythropoietin (EPO) production. Including numerous other factors, this inflammatory mechanism contributes to the development of cancer-related anaemia[6]. Furthermore, thrombocytosis and leucocytosis can also be induced by tumour derived cytokines or growth factors, which generate a pro-tumorigenic environment promoting angiogenesis[7-9]. Leucocytosis is frequently observed in obese patients, in whom adipose tissue in itself establishes a pro-inflammatory environment, stimulating carcinogenic cellular proliferation pathways. In this way, leucocytosis and obesity may relate to an adverse outcome in EC patients[10]. Furthermore, an association between high leucocyte count and high glucose level was found in female cancer patients, related to diabetes[11].

Several studies in EC have studied the relationship of pre-operative haematological parameters (anaemia, thrombocytosis and/or leucocytosis) with clinicopathological characteristics and the prognostic relevance[2,7-9,12-20]. However, only the study of

Njolstad et al. studied all three pre-operative haematological parameters, without specifying the correlation of anaemia, thrombocytosis and/or leucocytosis with clinicopathological characteristics[13]. Therefore, it remains unclear whether these parameters reflect tumour aggressiveness or merely indicate a poorer health status.

We hypothesise that anaemia and thrombocytosis reflect tumour aggressiveness, whereas leucocytosis may also be related to a poorer health status, including obesity and comorbidity, impacting survival in EC. Therefore, the aim of this study was to define the relationship of anaemia, thrombocytosis and leucocytosis with clinicopathological characteristics in EC patients. Furthermore, we aim to explore the prognostic relevance in terms of survival of these haematological parameters in EC patients, taking their comorbidities into consideration.

Materials and Methods

Study cohort

An observational multicentre cohort study was performed in women diagnosed with EC in 10 hospitals; nine in the Netherlands and one in the United Kingdom (UK; Royal Cornwall Hospital Trust). Patients who underwent surgical treatment in the nine Dutch participating centres were prospectively included between September 2011 until December 2013 in the PIpelle Prospective ENDOmetrial carcinoma (PIPENDO) study[1]. For the participating centre in the UK, data were collected retrospectively; the study population consisted of women who underwent surgical treatment for EC between January 2006 and January 2015.

Data collection

For the Dutch participating hospitals, patient characteristics (including age, Body Mass Index (BMI), diabetes mellitus (DM) and hypertension), post-operative tumour histology, grade and FIGO staging were collected prospectively. Pre-operative haemoglobin level, leucocyte- and platelet counts were collected retrospectively from hospital records. For the UK centre, all the clinicopathological characteristics and pre-operative haematological parameters were collected retrospectively. Patients were included if one of the haematological parameters was available for analysis. Furthermore, outcomes, including overall survival (OS) and disease-specific survival (DSS) were collected.

Definition pre-operative haematological parameters

Anaemia was defined as haemoglobin level <7.45mmol/L= <12g/Dl, thrombocytosis as platelet count >400x10^9 /L and leucocytosis as leucocyte count >10x10^9/L.

Outcome measurements

Defining the relationship of anaemia, thrombocytosis and leucocytosis with

clinicopathological characteristics in EC patients. Furthermore, to define the prognostic relevance in terms of survival of these haematological parameters in EC patients, taking into consideration their comorbidities (age, DM, BMI and hypertension).

Statistical analysis

For statistical analyses, Statistical Package for the Social Sciences (SPSS), version 25.0 (IBM, New York, NY, USA) was applied. The results were considered significant with p-value less than 0.05 ($p < 0.05$). A non-parametric Mann-Whitney U-test was used to compare continuous data of haematological values between categories. Differences between categories in haematological values and categories were compared with χ^2 or Fisher's exact test.

BMI was subdivided into five categories: BMI < 25, BMI 25 - 30, BMI 30 - 35, BMI 35 - 40, BMI > 40 kg/m^2. FIGO stage was divided binary; stage I-II (early-stage) and stage III-IV (advanced-stage).

Survival analyses were performed using Kaplan-Meier curves (first 10 year after diagnosis) and univariate and multivariate Cox-regression. Associations are shown as hazard ratio (HR), 95% Confidence Interval (CI) and p-value. OS was defined as time from date of diagnosis to date of death from any cause and DSS was defined as time from date of diagnosis to date of death by EC, all censored by date of last contact. OS and DSS were chosen as survival outcomes to compare overall mortality (i.e. while considering factors influencing survival such as comorbidity and BMI) with EC-related mortality.

Institutional Review Board approval

For the participating Dutch centres, this study was revised and approved by the local medical ethical committee Brabant on November 2018 (Protocol 1129, METC, Brabant).

For the participating UK centre, ethical approval was obtained through the London–Fulham Ethical committee on January 2015 (reference number 15/LO/0149) and the study had full hospital approval.

Results

Patients

A total of 920 patients with EC were included with a median follow-up of 4.5 years (range 0-10 years). Six patients were excluded; two patients based on abnormal leucocytosis (>50x10^9/L) caused by either chronic lymphatic leukaemia or by unknown cause, and four patients following final tumour pathology (no malignancy

of the uterus). Therefore, the final study population consisted of 914 patients.

Clinicopathological characteristics of the study cohort are shown in **Table 1**. The mean age of the study population was 66 years with median BMI 29.6 kg/m^2. Nearly half of all patients were diagnosed with hypertension (n = 433, 47.4%), and 152 patients (16.6%) with DM. Haemoglobin level was measured in 894 (97.8%) patients, platelet count in 721 (78.9%) and leucocyte count in 667 (72.9%) patients. Median haemoglobin level was 8.4 mmol/L, median platelet counts 298.3x10^9 platelets/L and median leucocyte level 8.1x10^9/L. Anaemia was present in 103 (11.3%) patients, thrombocytosis in 79 (8.6%) and leucocytosis in 114 (12.5%). Most patients were diagnosed with low-grade (grade 1-2) EC and endometrioid histology (71.6% and 83.5%, respectively). Lymphadenectomy was performed in 209 (22.9%) patients of whom 35 (16.7%) patients had lymph node metastasis. A total of 130 (14.2%) patients developed recurrent EC, and 163 (17.8%) patients were deceased of whom 101 (62.0%) directly related to EC.

Table 1. Clinicopathological characteristics

Patient characteristics		**Value (n = 914)**
Age (years)		66.2 (27.2-93.8)
BMI (kg/m^2)		29.6 (16.4-60.9)
Diabetes mellitus		152 (16.6)
Hypertension		433 (47.4)
Serum values		
Haemoglobin mmol/L		8.4 (3.9-10.6)
<7.45mmol/L		103 (11.3)
Platelets x10^9 /L		298.3 (13.9-781.0)
>400x10^9		79 (8.6)
Leucocytes x10^9/L		8.1 (2.2-33.5)
>10x109/L		114 (12.5)
Final tumour histology		
Tumour Grade	1-2	654 (71.6)
	3	256 (28.0)
	Not classified	4 (0.4)
Endometrioid		763 (83.5)
Non-endometrioid		151 (16.5)
	Serous	55 (36.4)
	Clear cell	15 (9.9)
	Mixed	74 (49.0)
	Others	7 (4.6)
FIGO stage	I-II	824 (90.2)
	IA	601 (65.8)
	IB	169 (18.5)
	II	54 (5.9)
	III-IVB	90 (9.8)
	IIIA	25 (2.7)
	IIIB	11 (1.2)
	IIIC	30 (3.3)
	IVA	7 (0.7)
	IVB	17 (1.9)

table continues

Patient characteristics		Value (n = 914)
Lymph node status		
	Positive (N1)	35 (3.8)
	Negative (N0)	174 (19.0)
	Unknown[1] (Nx)	705 (77.1)
Recurrence		
Yes		130 (14.2)
	Local	39 (4.3)
	Regional	26 (2.8)
	Distant	103 (11.3)
No		784 (85.8)
Mortality	Overall	163 (17.8)
	EC-related	101 (11.1)

Data is presented in numbers (%), mean or median (range)
Abbreviations: BMI = Body Mass Index (in kg/m^2), CA-125 = cancer antigen 125, FIGO = International Federation of Gynecology and Obstetrics, n = number
[1]no lymphadenectomy performed

Association of haematological parameters with clinicopathological characteristics

The correlations between anaemia, thrombocytosis and leucocytosis as well as their association with clinicopathological characteristics are shown in **Table 2**. There was a significant correlation between anaemia and thrombocytosis ($p < 0.001$) and thrombocytosis and leucocytosis ($p < 0.001$) (shown in detail in **Figure 1**). No association between anaemia and leucocytosis was found. Furthermore, anaemia, thrombocytosis and leucocytosis were significantly more prevalent in patients with advanced-stage disease (FIGO III-IV) ($p < 0.001$, $p < 0.001$ and $p = 0.030$, respectively) compared to patients presenting with early-stage (FIGO I-II) (**Figure 2**). In addition, anaemia was significantly more often observed in patients with grade 3 tumours ($p = 0.006$). Anaemia and thrombocytosis were significantly related to patient outcomes. Anaemia was related to distant recurrence, EC-related- and overall mortality ($p<0.001$ for all), and thrombocytosis associated with EC-related mortality ($p = 0.015$) (**Table 2**).

Association of haematological parameters with patient comorbidities

Pre-operative haemoglobin, leucocyte- and platelet count in relation to patients' characteristics are shown in **Table 3**. Leucocytosis was significantly associated with increased BMI ($p = 0.015$) and DM (24.3% vs 14.8%, $p = 0.031$, odds ratio (OR) 1.72 [CI 1.04-2.83]). In **Figure 3**, the percentage of patients with leucocytosis is shown for the overall patient population and the BMI subcategories. Only BMI 35-40 and >40 kg/m^2 were significantly associated with leucocytosis (OR 1.98 [CI 1.15-3.42] $p = 0.014$, OR 2.29 [CI 1.31-3.97] $p = 0.003$, respectively).

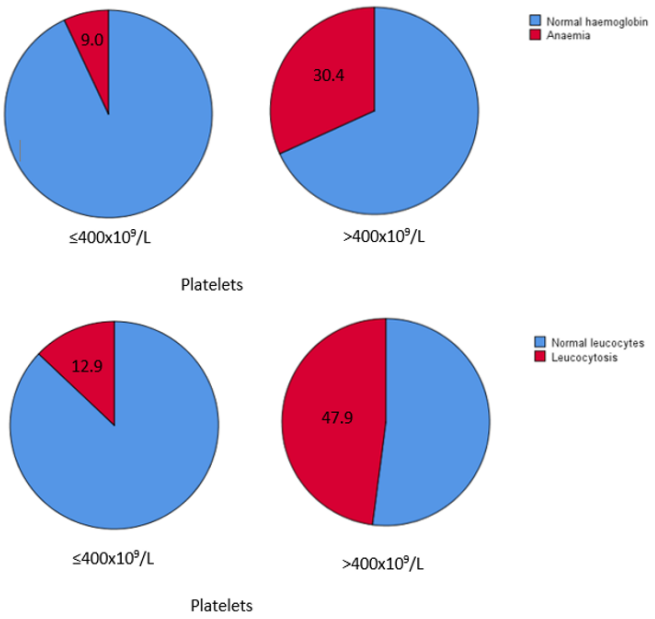

Figure 1. Percentage of patients with anaemia or leukocytosis distributed by platelet count

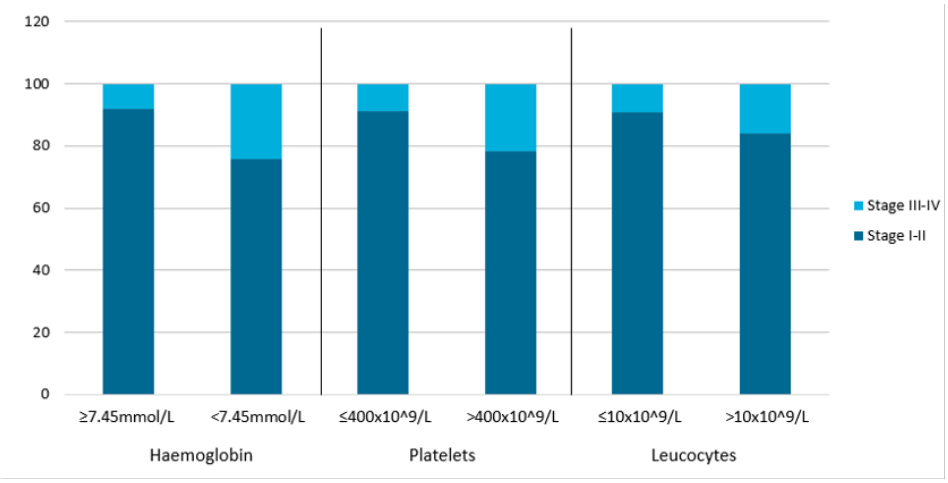

Figure 2. Correlation between haematological parameters and FIGO stage

Table 2. Clinicopathological characteristics in relation to haemoglobin-, leucocyte-, and thrombocytes-level

		Haemoglobin ≥7.45mmol/L (*n* = 791)	Haemoglobin <7.45mmol/L (n = 103)	p	Platelets ≤400x10^9/L (n = 642)	Platelets >400x10^9/L (n = 79)	p	Leucocytes ≤10x10^9/L (n = 553)	Leucocytes >10x10^9/L (n = 114)	p
Pre-operative parameters										
Haemoglobin <7.45mmol/L		-	-	-	58 (70.7)	24 (29.3)	**<0.001***	63 (78.8)	17 (21.2)	0.292
Platelets >400x10^9/L		55 (69.6)	24 (30.4)	**<0.001***	-	-	-	38 (52.1)	35 (47.9)	**<0.001***
Leucocytes >10x10^9/L		97 (85.1)	17 (14.9)	0.292	75 (68.2)	35 (31.8)	**<0.001***	-	-	-
Final tumour histology										
Tumour grade	1-2	575 (90.4)	61 (9.6)	**0.006***	461 (89.7)	53 (10.3)	0.438	397 (82.9)	82 (17.1)	0.911
	3	213 (83.9)	41 (16.1)		178 (87.7)	25 (12.3)		154 (83.2)	31 (16.8)	
Endometrioid		664 (89.4)	79 (10.6)	0.065	536 (89.6)	62 (10.4)	0.264	455 (82.9)	94 (17.1)	0.964
Non-endometrioid		127 (84.1)	24 (15.9)		106 (86.2)	17 (13.8)		98 (83.1)	20 (16.9)	
FIGO stage										
I-II		728 (90.3)	78 (9.7)	**<0.001***	587 (90.4)	62 (9.6)	**<0.001***	503 (84.0)	96 (16.0)	**0.030***
III-IV		63 (71.6)	25 (28.4)		55 (76.4)	17 (23.6)		50 (73.5)	18 (26.5)	
Outcome										
Recurrence		101 (80.2)	25 (19.8)	**0.002***	81 (86.2)	13 (13.8)	0.339	63 (76.8)	19 (23.2)	0.118
	Local	31 (81.6)	7 (18.4)	0.211	25 (89.3)	3 (10.7)	0.978	13 (61.9)	8 (38.1)	0.080
	Regional	20 (83.3)	4 (16.7)	0.480	12 (80.0)	3 (20.0)	0.222	10 (90.9)	1 (9.1)	0.266
	Distant	75 (75.0)	25 (25.0)	**<0.001***	64 (85.3)	11 (14.7)	0.178	54 (79.4)	14 (20.6)	0.603
Mortality		124 (77.5)	36 (22.5)	**<0.001***	113 (86.3)	18 (13.7)	0.260	87 (77.0)	26 (23.0)	0.067
EC-related mortality		74 (74.7)	25 (25.3)	**<0.001***	64 (81.0)	15 (19.0)	**0.015***	51 (75.0)	17 (25.0)	0.068

Data is presented in numbers (%)
Abbreviations: FIGO = International Federation of Gynecology and Obstetrics, n = number
** p < 0.05*

Table 3. Patient characteristics in relation to haemoglobin-, leucocytes- and platelet levels

	Age (years)	p	BMI (kg/m²)	p	Diabetes mellitus	p	Hyper-tension	p
Haemoglobin								
≥7.45mmol/L	66.3 ± 9.7	0.481	30.9 ± 6.9	0.701	127 (85.2)	0.174	372 (87.9)	0.635
<7.45mmol/L	67.2 ± 13.1		30.6 ± 7.9		22 (14.8)		51 (49.5)	
Platelets								
≤400x10⁹/L	66.7 ± 10.0	**0.008***	31.2 ± 7.1	0.256	108 (90.0)	0.713	319 (49.5)	0.276
>400x10⁹/L	63.5 ± 11.1		30.2 ± 6.8		12 (10.0)		34 (43.0)	
Leucocytes								
≤10x10⁹/L	66.6 ± 10.2	0.229	30.7 ± 6.8	**0.015***	81 (75.7)	**0.031***	266 (81.1)	0.222
>10x10⁹/L	65.2 ± 9.4		32.7 ± 7.9		26 (24.3)		62 (18.9)	

Data is presented in numbers (%), mean ± SD and median ± IQR
Abbreviations: BMI = Body Mass Index
** p < 0.05*

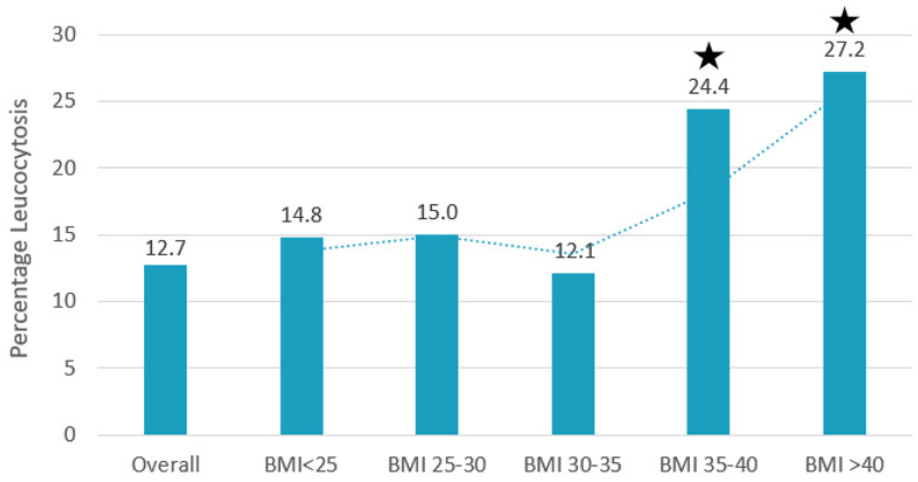

Figure 3. Overall patient population and Body Mass Index subgroups in relation to leucocytosis

Pre-operative haematological parameters and their association with survival outcomes
Explorative survival outcomes with all haematological parameters, individual and combined, are shown in Supplementary Figure 1A-B. Patients with anaemia, thrombocytosis and leucocytosis (n = 10) combined showed significantly poorer 10-year OS and DSS (60% and 70% respectively) compared to patients with

normal levels of haemoglobin, thrombocytes and leucocytes (n = 456) (86.2% and 92.8% respectively). Anaemia showed to be the most important prognosticator.

Detailed 10-year survival of anaemia and thrombocytosis is demonstrated in survival curves **Figure 4A-B**. Patients with anaemia or both anaemia and thrombocytosis showed comparable poor 10-year OS and DSS (67.2% vs. 66.7% and 77.6% vs 75.0% respectively). The detailed 10-year survival of leucocytosis and anaemia is shown in **Figure 4C-D.** Patients with anaemia and leucocytosis had the worst 10-year OS of 64.7%, compared to DSS (76.5%).

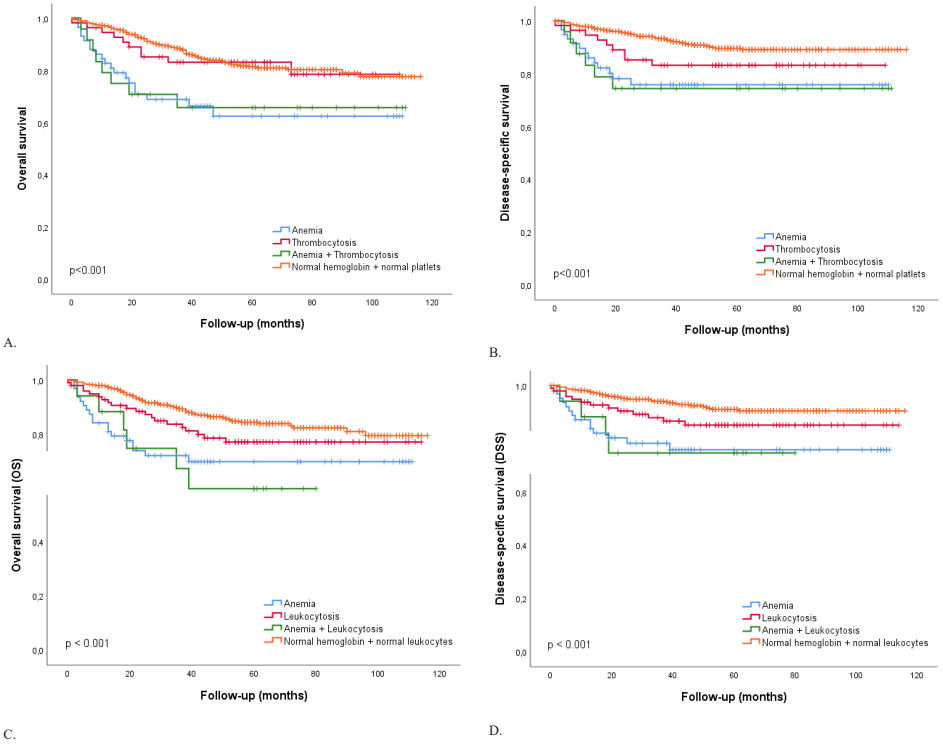

Figure 4. Kaplan-Meier survival curves of anaemia and thrombocytosis and anemia and leukocytosis

A. Overall survival for anaemia with and without thrombocytosis.

B. Disease-specific survival for anaemia with and without thrombocytosis.

C. Overall survival for anaemia with and without leucocytosis.

D. Disease-specific survival for anaemia with and without leucocytosis.

Women with leucocytosis and comorbidities (DM / hypertension / age ≥ 65 years / age ≥ 70 years / BMI ≥ 30 kg/m^2 / BMI ≥ 35 kg/m^2 / BMI ≥ 40 kg/m^2) did not

have a significantly worse survival compared to women with leucocytosis without comorbidities (*data not shown*). Only patients with leucocytosis and normal BMI (BMI < 25 kg/m^2) showed significantly lower 10-year DSS of 85% (p = 0.041) compared to all patients with leucocytosis (*data not shown*).

Prognostic relevance of pre-operative haematological parameters

Univariate analysis showed significantly decreased OS and DSS for patients with anaemia, and significantly decreased DSS for patients with thrombocytosis (**Table 4-5**).

Table 4. Cox regression analysis univariate and multivariate of overall survival

Variable	Univariate OS			Multivariate OS		
	HR	95% CI of HR	p-value	HR	95% CI of HR	p-value
Patient characteristics						
BMI ≥ 30	0.89	0.65-1.22	0.495			
Age ≥ 65	2.89	2.03-4.13	**<0.001***	2.94	2.01-4.29	**<0.001***
Diabetes Mellitus	1.63	1.13-2.35	**0.008***	1.39	0.95 -2.04	0.090
Hypertension	1.56	1.14-2.14	**0.005***	1.18	0.84-1.66	0.321
Haematological parameters						
Anaemia	2.73	1.89-3.96	**<0.001***	2.29	1.55-3.37	**<0.001***
Leucocytosis	1.49	0.96-2.31	0.085			
Thrombocytosis	1.27	0.77-2.10	0.336			
Post-operative histology						
Histology - grade 3 tumour	4.30	3.09-5.97	**<0.001***	2.69	1.90-3.83	**<0.001***
FIGO stage						
I-II vs III-IV	9.34	6.75-12.91	**<0.001***	5.49	3.79-7.95	**<0.001***

Abbreviations: CI = Confidence Interval, HR = Hazard Ratio, OS = Overall Survival
** p < 0.05*

Table 5. Cox regression analysis univariate and multivariate of disease-specific survival

Variable	Univariate DSS			Multivariate DSS		
	HR	95% CI of HR	p-value	HR	95% CI of HR	p-value
Patient characteristics						
BMI ≥ 30	0.74	0.49-1.10	0.138			
Age ≥ 65	2.58	1.65-4.00	**<0.001***	2.68	1.57-4.58	**<0.001***
Diabetes Mellitus	0.98	0.57-1.67	0.945			
Hypertension	1.42	0.95-2.09	0.083			
Haematological parameters						
Anaemia	3.16	2.00-4.99	**<0.001***	1.84	1.04-3.26	**0.034***
Leucocytosis	1.66	0.95-2.87	0.072			
Thrombocytosis	1.91	1.09-3.35	**0.024***	1.33	0.72-2.45	0.355
Post-operative histology						
Histology - grade 3 tumour	8.54	5.33-13.71	**<0.001***	4.65	2.64-8.19	**<0.001***
FIGO stage						
I-II vs III-IV	17.12	11.52-25.44	**<0.001***	7.79	4.73-12.84	**<0.001***

Abbreviations: CI = Confidence Interval, HR = Hazard Ratio, DSS = Disease Specific Survival
** p < 0.05*

In multivariate analysis, age ≥65 years, DM, anaemia, grade 3 tumour and advanced-stage disease (FIGO III-IV) were independently associated with reduced OS (**Table 4**). Age ≥65 years, anaemia, grade 3 tumour and advanced-stage disease (FIGO III-IV) were independently associated with reduced DSS (**Table 5**).

Discussion

Principal findings

To our knowledge, this is the largest study to date investigating the relationship of pre-operative haematological parameters with clinicopathological characteristics and survival in EC. Preoperative anaemia and thrombocytosis are significantly associated with advanced-stage disease. Moreover, anaemia is associated with thrombocytosis and grade 3 tumour. EC patients with anaemia or both anaemia and thrombocytosis showed comparable poor survival (OS and DSS). Furthermore, anaemia was identified as an independent prognostic factor for both OS and DSS, in addition to age ≥ 65 years, grade 3 tumour and advanced-stage disease. Pre-operative leucocytosis was significantly associated with increasing BMI, DM and advanced-stage disease, yet did not show a significant poorer survival in EC patients.

Results

A significantly higher rate of anaemia in patients with advanced-stage disease was found, in line with earlier published data[12,14,15]. The relation between anaemia and grade 3 tumour supports the overall more aggressive tumour behaviour in these grade 3 tumours. Previous studies also support the association between anaemia and thrombocytosis as found in our study[12,15]. This correlation may be explained by the involvement of erythropoietin (EPO) in the development of thrombocytosis that is associated with cancer-related anaemia. Increased EPO levels result in an increase of megakaryocyte maturation which causes increased platelet production[21]. As a consequence, the common aetiology of anaemia and thrombocytosis may explain our observed relation between thrombocytosis and advanced-stage disease, and is in line with three recent meta-analyses[9,16,17]. The studies by Bai et al. and Nie et al. showed a significant association between thrombocytosis and clinicopathological characteristics, in particular for advanced-stage disease[9,16]. Although increased EPO levels may partly explain the occurrence of anaemia and thrombocytosis, further mechanisms are not fully elucidated. Some studies hypothesised that platelets infiltrate tumour tissue and contribute to tumour growth by secreting pro-tumorigenic factors and pro-angiogenic factors, while others suggested a platelet-cancer interaction is formed to facilitate cancer cell migration, which contributes cancer metastasis[22-24].

Furthermore, a correlation between leucocytosis and advanced-stage disease was found, similar to previous studies[2,8,18,20]. This mechanism may be related by

upregulation of inflammatory cytokines and hematopoietic growth factor through tumour cells and promoting enhanced inflammation, leucocytosis, angiogenesis and tumour cell proliferation[5,25]. Njolstad et al. studied all three pre-operative haematological parameters and showed an association between advanced-stage EC and lower haemoglobin levels, elevated platelet- and elevated leucocyte counts. Yet, in specifically this analysis, they did not further dichotomize into anaemia, thrombocytosis and leucocytosis[13]. Our findings support the theory that pre-operative anaemia, thrombocytosis as well as leucocytosis may reflect tumour aggressiveness in EC.

Besides the increased leucocyte levels in advanced-stage EC, we hypothesised leucocytosis to be a poor prognostic factor especially in patients with inflammatory-related comorbidities such as DM and obesity. However, while women with DM and obesity were more likely to show leucocytosis[10,11], leucocytosis was not an independent prognostic factor in EC, and even combined with obesity or DM it did not impact outcome.

Pre-operative haematological parameters also appear relevant in survival outcomes of EC[8,9,14-18,20]. In our study, anaemia was an independent prognostic factor in multivariate analyses, which has not been reported by previous studies[14,15]. However, our study included the largest number of patients to date (n = 914 vs. n = 61 and n = 228). Three recently performed meta-analyses, including 2500-3500 EC patients, reported conflicting results regarding pre-operative thrombocytosis and outcome[9,16,17]. Our study showed no reduced OS for patients with thrombocytosis, which is in line with two meta-analyses[16,17]. We did, however, show significant reduced univariate DSS, in line with two previous studies also included in meta-analyses[13,26]. Furthermore, the study of Worley et al. described leucocytosis as an independent prognostic factor for EC[8], however our study and the recent study of Salem et al. did not replicate this finding[20].

Pre-operative haematological parameters may be used separately or combined to increase prognostic significance. Njolstad et al. showed significantly worse DSS in patients with anaemia, leucocytosis and thrombocytosis combined, which is in line with our results[13].

Clinical implications

Patients with abnormal pre-operative haematological parameters should be identified as high-risk patients, who may require an extended diagnostic work-up or a possible refinement of therapy including extensive staging procedures and/or adjuvant therapy.

Research implications

Future research in prospective trials should be performed by focusing on novel treatment strategies for EC patients with abnormal pre-operative haematological parameters. For example, examining the effect of surgical staging and/or adjuvant therapy in terms of survival on EC patients with abnormal haematological parameters compared to EC patients with normal haematological parameters. Furthermore, our study did not investigate the metabolic syndrome, this could be a next step, in which identification of patients with metabolic syndrome pre-operatively warrants further treatment of metabolic syndrome post-operatively.

Limitations and strength

Our results may be limited by the small number of patients who underwent complete surgical staging including lymphadenectomy (22.8%). This could have influenced our results of anaemia as good prognosticator, however survival analyses in patients with early-stage disease (FIGO I-II) still showed shorter OS and DSS for patients with anaemia (*data not shown*). Therefore, we believe this has only minimal impact on our study results. Another limitation is the partly retrospective collection of data; inherent to the retrospective design of the study, haemoglobin level, platelet- and leucocyte count were not available for each included patient.
To our knowledge, we are the first to study the relationship of all three pre-operative haematological parameters with clinicopathological characteristics and prognostic outcome in EC. Other strengths of this study include its multicentre design, the largest patient cohort to date, and the well-documented and long follow-up period.

Conclusion

In conclusion, pre-operative anaemia, thrombocytosis and leucocytosis are related to tumour aggressiveness in EC. Anaemia has the most important prognostic relevance, and could occur with thrombocytosis. Leucocytosis appears more related to obesity and comorbidity and reflects a poorer health status, without impacting survival.

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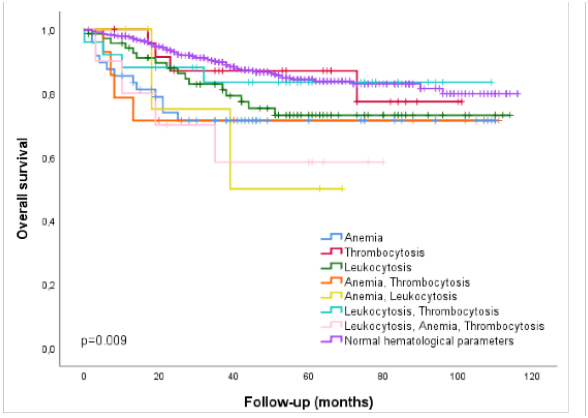
Supplemental material

A.



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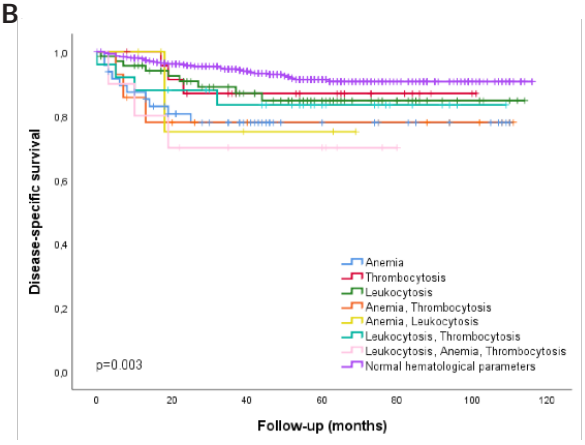
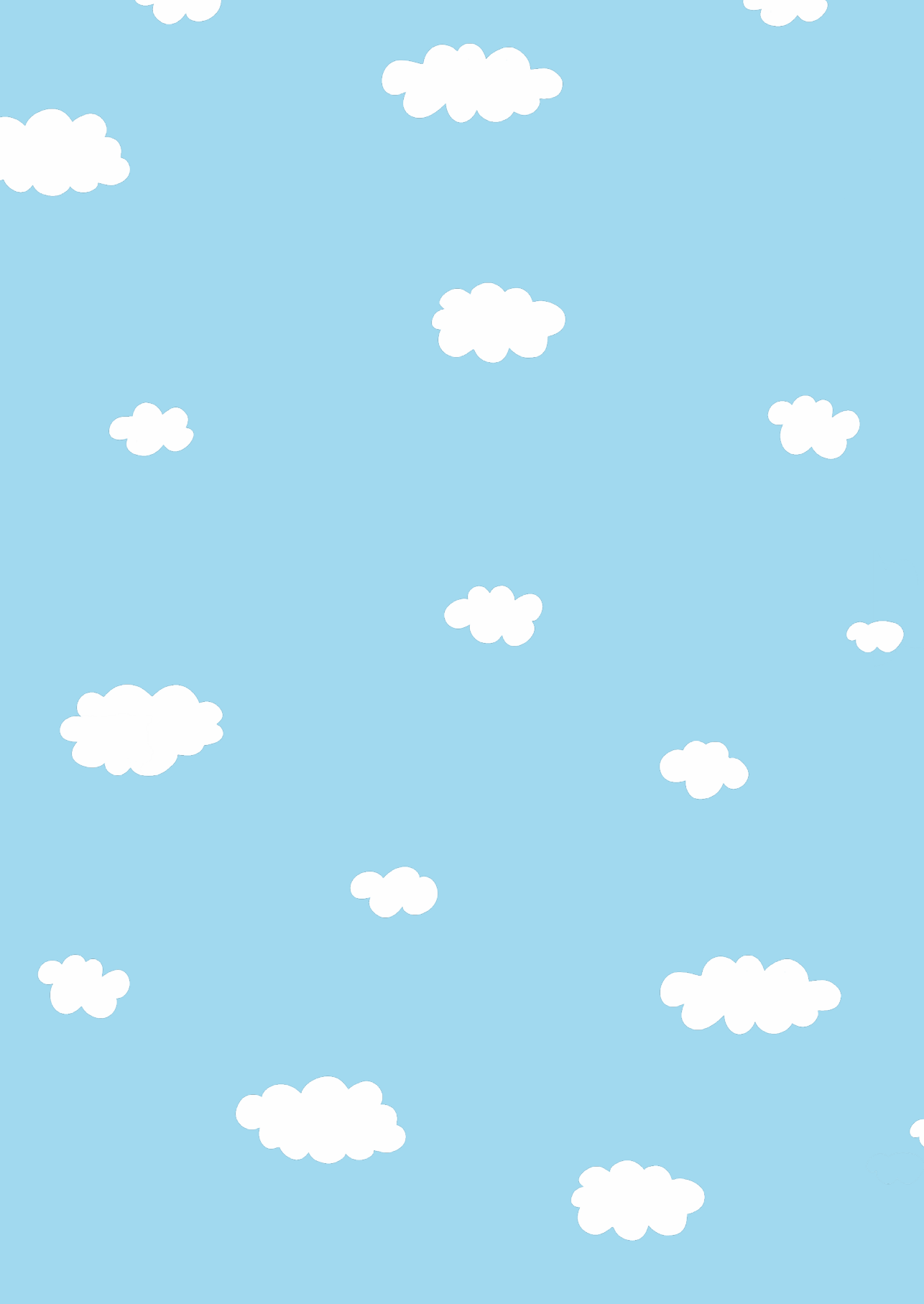


Supplementary Figure 1. Kaplan-Meier survival curves of the haematological parameters
A. Overall survival of anaemia, thrombocytosis and leucocytosis, separately and combined.
B. Disease-specific survival of anaemia, thrombocytosis and leucocytosis, separately and combined.

Part II

A novel diagnostic biomarker in the early detection of endometrial cancer



Chapter 7

Diagnostic value of microRNA panel in endometrial cancer: A systematic review

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Oncotarget.
2020 May 26;11(21):2010-2023

Abstract

Purpose
We conducted a systematic review to evaluate the overall diagnostic accuracy of microRNAs (miRNAs) in detecting endometrial cancer (EC).

Methods
A systematic search of Medline, Embase, Cinahl and the Cochrane Controlled Register of Trials was performed to identify studies reporting on the diagnostic value of miRNA in EC patients. Included were diagnostic studies looking at miRNA expression in women diagnosed with EC. Two reviewers independently selected studies and assessed quality of studies using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) score system. Data extraction was completed and the vote-counting strategy was used to rank miRNAs.

Results
26 studies were included with a total number of 1,400 EC patients reporting on 106 differentially expressed miRNAs. The most frequently found up-regulated miRNA was miR-205 followed by miR-200c, -223, -182, -183 and -200a. In addition, miR-135b, miR-429, miR-141 and miR-200b were also frequently up-regulated. There was less consensus on down-regulated miRNAs.

Conclusion
MiRNAs yield a promising diagnostic biomarker potential in EC, especially miR-205, the miR-200 family and miR-135b, -182, -183 and -223. However, no sufficient high-quality data are available to draw hard conclusions. More research is needed to validate the diagnostic potential of these miRNAs in larger studies. In addition, the potential of urine as a non-invasive biofluid should be investigated in more detail.

Introduction

Endometrial cancer (EC) is the most common malignancy of the female genital tract and the 8th cause of death in women in the United Kingdom (UK)[1]. Two different subtypes of EC have been described: type I tumours are mostly endometrioid adenocarcinomas and are associated with unopposed oestrogen stimulation and obesity and are often preceded by endometrial hyperplasia[2]. Type II tumours on the other hand are predominantly serous carcinomas, are commonly described as oestrogen independent arising in atrophic endometrium and are less well differentiated and therefore haver poorer prognosis[2,3]. The large majority of endometrial cancer are type I endometrioid, which is associated with good prognosis[4]. This is largely because women present early with bleeding problems and are therefore diagnosed at an early stage[5]. However, between 15 to 25% of women present with advanced stage disease (stage III or stage IV) with a 5-year survival varying from 40% to 79% for FIGO stage III, and from 0% to 24% for FIGO stage IV disease[6].

At the moment, diagnosis of EC is made by combination of transvaginal ultrasound scan (TVUS) and endometrial biopsy, which is an invasive and uncomfortable investigation with Visual Analogue Scale (VAS) pain score of 6.5 in postmenopausal women[7,8]. In addition, high numbers of technical problems (12-23%) and insufficient amount of tissue (16-68%) in obtaining endometrial biopsy have been described[9]. Therefore, the identification of validated and non-invasive diagnostic biomarkers are needed. These biomarkers need to be accurate in order to improve earlier diagnosis and outcomes including survival.

MicroRNAs (miRNAs) are small noncoding RNAs involved in posttranscriptional regulations of various cellular processes and over 2,000 human miRNAs are identified[10,11]. MiRNAs have been demonstrated to play a major role in a wide range of developmental processes including metabolism, cell proliferation, apoptosis and developmental timing[12]. Overexpressed miRNAs may function as both oncogenes (through downregulation of tumour-suppressor genes) and/or regulator of cellular processes such as cell differentiation or apoptosis. This is thought to be how miRNAs are associated with the development of different cancer types such as colorectal, breast, ovarian and endometrial cancer[13–16], although the exact pathways are not entirely understood. Because of their potential role as agents controlling cell growth and differentiation, miRNAs have been proposed to be good candidates for cancer diagnosis and therapy[17]. In addition, previous systematic reviews show a promising diagnostic potential of miRNA in cancer types such as ovarian and pancreatic cancer[18,19], however the diagnostic value of miRNA for endometrial cancer remains unclear. Results of published studies are inconsistent due to differences in study design, specimen types and miRNAs and

different groups have obtained conflicting conclusions.

MiRNAs can be detected in fixed tissue specimens but also in blood, serum, urine and other body fluids[20]. To date it is unclear which specimen type can be used to achieve the most reliable and feasible biomarker for the detection of EC.

Therefore, this systematic review was conducted to summarize the global research and to evaluate the potential diagnostic value of miRNAs in detecting endometrial cancer. The aim of this systematic review is to provide guidance for future researchers as to which aspects of miRNA expression in EC warrants further exploration.

Materials and methods

Search strategy

This review was performed according to Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines, and in accordance with the principles outlined in the Cochrane Handbook[21]. Systematic searches were performed in Medline (1946 until May 2019), Embase (1980 until May 2019) and Cinahl (1981 until May 2019) and the Cochrane Controlled Register of Trials with the following terms: ('microRNA' OR 'miRNA' OR 'miR') AND ('endometrial cancer' OR 'endometrial carcinoma' OR 'endometrial neoplasm' OR 'uter* carcinoma' OR 'uter* cancer' OR 'uter* neoplasm'). Search strategies were adapted accordingly to each database. In addition, grey literature was searched including abstracts of scientific meetings as well as manually checking the reference lists of eligible studies to identify any additional studies to include in this review.

Study inclusion/exclusion criteria

Studies were considered eligible if publications met all the following criteria: (1) the study concerned the diagnostic value of miRNAs; (2) histological subtype was specified as primary endometrial cancer; (3) studies used real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) techniques to detect miRNA expression, (4) the study was in English; (5) the study was conducted in human subjects; (6) the study was not a review, abstract or editorial article. Cell line models were excluded due to the limitations cell lines models have in terms of the in vitro adaptation of cells to culture conditions, which sometimes leads to the discrepancy between the experimental and clinical outcomes[22].

Study selection

Two reviewers (HD and KG) independently assessed titles and abstracts of all identified studies. Those studies that clearly did not meet the inclusion criteria were excluded. Potentially relevant studies were retrieved in full text and were

further reviewed for eligibility by both reviewers.

Data extraction

Data extraction was completed by two reviewers (HD and KG) and disagreements were resolved by consensus. The necessary information and data were extracted from the final eligible articles as follows: first author, year of publication, country of origin, number of cases and controls, histology type, miRNA expression test methods, type of specimens, cut-off values, expression changes and sensitivity and specificity.

Trial quality assessment

The methodological qualities of the selected eligible articles were assessed by the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) score system[23]. The QUADAS-2 tool combines the patient selection index, index test, reference standard, flow and timing to evaluate risk of bias and applicability. The seven items (four items on risk of bias and three items on applicability) were assessed for all included articles. Two authors independently tested the pilot QUADAS-2 items (HD and KG) and discrepancies were resolved by consensus.

Ranking of miRNA

In order to collect and sum up the results of the included studies, the vote-counting strategy was used[24]. The vote-counting strategy ranks biomarkers on the basis of one principal and two secondary criteria and is the most common and most frequently cited strategy to rank biomarker candidates systematically[24,25]. The principal criterion is made up of the number of studies in which each study showing significant differential expression in the same direction (either up- or down-regulated) for a biomarker counts as a vote in favour of that biomarker being real. The secondary criteria are (1) total sample size summed across all of the supporting studies (the assumption being that larger studies tend to be more reliable) and (2) mean fold change (based on the idea that large differences in biomarker expression are more likely to be confirmed than small differences).

Results

Study selection

The flow diagram of the selected studies is depicted in **Figure 1**.

The initial literature search identified 3,253 articles, from which 42 duplicates were excluded. Of the remaining 3,211 articles, 3,142 were excluded based on title and abstract screening. The search identified 69 full texts, of which 43 articles were excluded for the following reasons:

- In vitro studies (using cell lines)
- Not diagnostic
- Only included patients after adjuvant radiotherapy
- Compared EC with ovarian cancer
- Did not have a comparison group
- Abstracts only
- Only focused on sarcomas
- Focused on the prediction of lymph node metastasis and miRNA expression

In addition, no other potential articles from the references of other reviews in the full-text screening process were found. Finally, 26 articles were included in this systematic review.

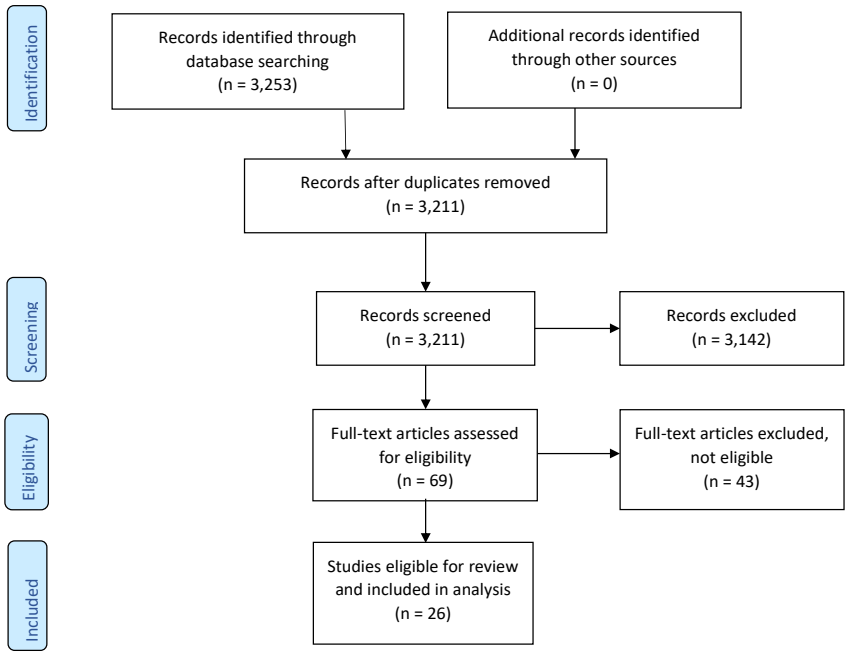

Figure 1. PRISMA flow diagram

Study characteristics and quality assessment

The principal characteristics of the included studies are outlined in **Table 1**.

Table 1. The main characteristics of included studies

Author	Country	Subtype	Sample size *Cancer*	*Hyperplasia*	*Controls*	Specimen	Test method	Outcome**	Sensitivity / specificity
Hiroki et al. (2010)	Japan	Serous	21	-	7	FFPE	Microarray and RT-qPCR	Up-regulated: miR-205 Down-regulated: miR-101, miR-10b*, miR-133a, miR-133b, miR-152, miR-29b, miR-34b, miR-411	-
Jayaraman et al. (2017)	USA	Not specified	49	-	6	FFPE	RT-qPCR	Up-regulated: miR-141-3p, miR-96-5p Down-regulated: miR-26a-5p, miR-150-5p, let-7f-5p, miR-26b-5p, let-7c-5p, miR-125a-5p, miR-195-5p, miR-23b-3p, miR-374a-5p, miR-126-3p, miR125b-5p, miR-424-5p, let-7-a-5p, let-7e-5p	-
Torres et al. (2012)	Poland	Endometrioid	**Fresh frozen** 30 **FFPE** 43 **Plasma** 34	**Fresh frozen** - **FFPE** - **Plasma** -	**Fresh frozen** 16 **FFPE** 15 **Plasma** 14	Fresh frozen, FFPE and plasma	Microarray and RT-qPCR	**Tissue:** Up-regulated: miR-9, miR-92a, miR-96, miR-135b, miR-182, miR-183, miR-200a, miR-200a*, miR-200b, miR-200b*, miR-200c, miR-203, miR-205, miR-429 Down-regulated: miR-410, miR-1305	**Tissue:** miRNA signature miR-92a/miR-410 AUC 0.977 and miR-92a/miR-205/miR-410 AUC 0.984. **Plasma:** miRNA signature miR-9/miR-1228 AUC 0.909 and miR-9/miR-92a AUC 0.913

table continues

Author	Country	Subtype	Sample size *Cancer*	*Hyperplasia*	*Controls*	Specimen	Test method	Outcome**	Sensitivity / specificity
								Plasma: Up-regulated: miR-92a, miR-141, miR-200a, miR-203, miR-449a, miR-1228, miR-1290 Down-regulated: miR-9, miR-301b	
Karaayvaz et al. (2012)	USA	All sub-types	48	-	5	FFPE	Microarray and RT-qPCR	Up-regulated: miR-200c, miR-205	-
Tsukamoto et al. (2015)	Japan	Endome-trioid	**Fresh tissue** 28 **Plasma** 12	**Fresh tissue** - **Plasma** -	**Fresh tissue** 14 **Plasma** 12	Fresh tissue samples and plasma	Next-generation sequencing and RT-qPCR	**Tissue:** Up-regulated: miR-499, miR-135b, miR-205 Down-regulated: miR-10b, miR-195, miR-30a-5p, miR-30a-3p, miR-21 **Plasma:** Up-regulated: miR-135b, miR-205 Down-regulated: mi-30a-3p and miR-21	**Tissue:** miRNA signature miR-135b/miR-195 AUC 0.9835 and miR-135b/mi-R30a-3p AUC 0.9898 **Plasma:** AUC for miR-21: 0.7569, AUC for miR-30-a-3p: 0.8125 AUC for miR-135b: 0.9722 AUC for miR-205: 1.0
Wilczynski et al. (2018)	Poland	Endome-trioid	90	-	10	FFPE	RT-qPCR	Up-regulated: miR-200c	-
Fang et al. (2018)	China	Not specified	176	-	100	Serum	RT-qPCR	Down-regulated: miR-93	AUC: 0.781 (0.724-0.842)

Kottaridi et al. (2017)	Greece	Not specified	**FFPE** 16 **LBC** 12	**FFPE** 8 **LBC** 6	**FFPE** 34 **LBC** 28	FFPE and liquid based cytology (LBC)	RT-qPCR	**FFPE:** Up-regulated: miR-9-5p, miR141-3p, miR182-5p, miR-200b-3p, miR-200c-3p, miR205-5p **LBC:** Up-regulated: miR-182-5p, miR-141-3p, miR-200b-3p, miR-200c-3p, miR-205-5p, miR-222-3p	**FFPE:** miR-182-5q ROC 0.979, 4-fold overexpression sensitivity 93.8%, specificity over 95% miR-141-3p and 13-fold increase AUC 0.964, sensitivity 81.3% miR-205-5p 8-fold increase AUC 0.964, sensitivity 81.3% **LBC** similar results with lower AUCS; miR-141-3p 0.955, miR-182-5 0.942 with sensitivity of 85.7% and specificity of 100% for 5-fold increase.
He et al. (2017)	China	All types	68	-	20	Liquid nitrogen and FFPE	RT-qPCR	Up-regulated: miR-944	-
Jiang et al. (2016)	China	Not specified	50	-	50	Serum	RT-qPCR	Up-regulated: miR-887-5p	AUC for EC diagnosis 0.728, specificity 0.60, sensitivity 0.95 (0.563-0.892)
Benati et al. (2017)	Italy	Clear cell, endometrioid and serous	45	-	30	Serum	RT-qPCR	Up-regulated: miR-203	AUC 0.71

table continues

Author	Country	Subtype	Sample size *Cancer*	*Hyperplasia*	*Controls*	Specimen	Test method	Outcome**	Sensitivity / specificity
Srivastava et al. (2018)	USA	Not specified	22	-	5	Urine	RT-qPCR	Up-regulated: miR-200c-3p	-
Lu et al. (2016)	China	All types	67	-	15	Snap-frozen samples	Microarray and RT-qPCR	Up-regulated: miR-21, miR-196a, miR-16, miR-582-5p, miR-15b, miR-301, miR-148b, miR-128a Down-regulated: miR-125, miR-34	-
Wang et al. (2014)	China	Endometrioid	40	4	49	Plasma	RT-qPCR	Up-regulated: miR-27a, miR-15b, miR-223	AUCs: 0.768 for miR-15b; 0.813 for miR-27a; 0.768 for miR-223. Combining miR-27a and CA125: AUC 0.894
Lee et al. (2013)	Korea	Endometrioid	**FFPE** 20 **Fresh frozen** 4	**FFPE** - **Fresh frozen** -	**FFPE** 10 **Fresh frozen** 4	FFPE and fresh frozen tissue	Microarray and RT-qPCR	Up-regulated: miR-200a*, miR-205, miR-141, miR-200b*, miR-182	-
Wilczynski et al. (2016)	Poland	Endometrioid	90	-	10	FFPE	RT-qPCR	Up-regulated: miR-205	-
Jia et al. (2013)	China	Endometrioid	26	-	22	Serum	RT-qPCR	Up-regulated: miR-222, miR-223, miR-186, miR-204	ROC curve of four-serum miRNA signature was 0.927 (individual ones ranging from 0.727 to 0.837).

									AUC of 0.927, specificity 87.5% and sensitivity 91.7%
Cohn et al. (2010)	USA	All types	141	-	20	FFPE	Microarray and RT-qPCR	Up-regulated: miR-200c, miR-183, miR-205, miR-223 and miR-425	Cluster sensitivity 92% and positive predictive value 97%
Lee et al. (2012)	Korea	Endometrioid	22	43	10	FFPE	RT-qPCR	Up-regulated: miR-182, miR-183, miR-200a, miR-200c, miR-205	Composite panel of six miRNAs sensitivity 91% and specificity 94% Individual 64-77% sensitivity and 66-91% specificity.
Snowdon et al. (2011)	Canada	Endometrioid	14	10	10	FFPE	Microarray and RT-qPCR	Up-regulated: miR-200a, miR-429 Down-regulated: miR-503, miR-542-5p	-
Montagnana et al. (2017)	Italy	Not specified	46	-	28	Serum	RT-qPCR	Up-regulated: miR-186, miR-222, miR-223 Down-regulated: miR-204	-
Jurcevic et al. (2016)	Sweden	Endometrioid	30	-	20	FFPE	RT-qPCR	Up-regulated: miR-183, miR-182, miR-429, miR-200b, miR-200a, miR-141, miR-18a, miR-200c, miR-18a*, miR-106a, miR-17, miR-34a, miR-92a-1*, miR-106b*, miR-20a*, miR-17*, miR-185 Down-regulated: miR-1247, miR-376c, miR-377, miR-370, miR-214, miR-337-5p, miR-300, miR-758	-

table continues

Author	Country	Subtype	Sample size *Cancer*	*Hyperplasia*	*Controls*	Specimen	Test method	Outcome**	Sensitivity / specificity
Chung et al. (2009)	Hong Kong	Endometrioid	38	-	28	Snap-frozen tissue	RT-qPCR	Up-regulated: miR-205, miR-182, miR-200a, miR-223, miR-210, miR-200c, miR-183, miR-155, miR-203, miR-194, miR-95, miR-106a, miR-103, miR-151	-
Al-Deresawi et al. (2018)	Iraq	Endometrioid	60	-	10	Tissue specimen	RT-qPCR	Down-regulated: miR-203	-
Devor et al. (2017)	USA	Endometrioid and serous	23	-	4	Snap-frozen tissue	RT-qPCR	Up-regulated: miR-135a, miR-135b, miR-200c, miR-205 Down-regulated: miR-137, miR-129-3p	-
Torres et al. (2012)	Poland	Endometrioid	**Tissue** 73 **Plasma** 4	-	**Tissue** 31 **Plasma** 14	Fresh frozen tissue and FFPE	RT-qPCR	**Tissue:** Down-regulated: miR-99a, miR-100, miR-199b **Plasma:** Up-regulated: miR-99a, miR-100, miR-199b	AUC miR-199b tissue 0.704. AUC miR-99a plasma 0.810. 2-miRNA signature based on miR-99a and miR-199b in plasma sensitivity 88% and specificity 93%

** *MiRNAs only reported in table if detected or confirmed with PCR.*

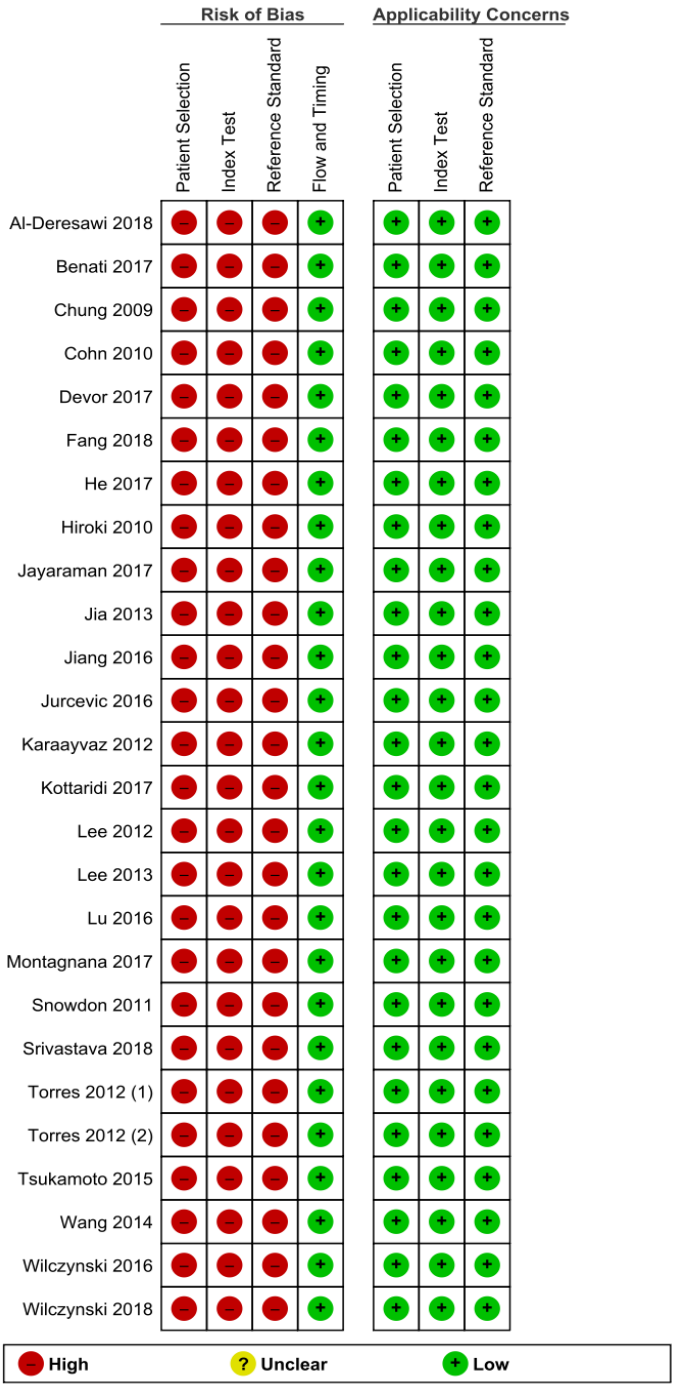

Figure 2. Summary of bias risk assessment results for QUADAS-2

In this review, 26 articles were included with a total of 2,110 women of which 1,400 had EC, 71 had either simple or complex atypical hyperplasia and 639 women had benign endometrium or polyps. The majority of studies were conducted in Asia (12 articles), 8 in Europe and 6 articles were conducted in United States/Canada. There were 16 studies that detected miRNAs in tissue specimens (Formalin-Fixed, Paraffin-Embedded (FFPE) or fresh frozen tissue), 5 studies that used serum[15,26–29], 1 study detected miRNA in plasma[30] and 1 study used urine as bio-fluid [31]. Two studies used both tissue and plasma samples and one study used both liquid based cytology (LBC) endometrial samples and tissue samples[32]. The majority of the studies only included EEC (13 articles), 6 did not specify which subtype they included and 5 articles included both subtypes of EC (EEC and NEEC)[15,33–36]. Furthermore, 1 article only included serous endometrial carcinomas[37] and 1 article included both serous endometrial carcinomas and EEC but no other EC subtypes[38]. All studies used real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) methods to detect miRNA expression, either solely or after microarray or Northern Blot analysis. The risk of bias and applicability of these studies were evaluated based on QUADAS-2 and summarized in **Figures 2** and **3**. There was a high risk of bias in all studies on patient selection, index test and reference standard but a low risk of bias for flow and timing. Furthermore, there were no applicability concerns in any of the studies included in this review.

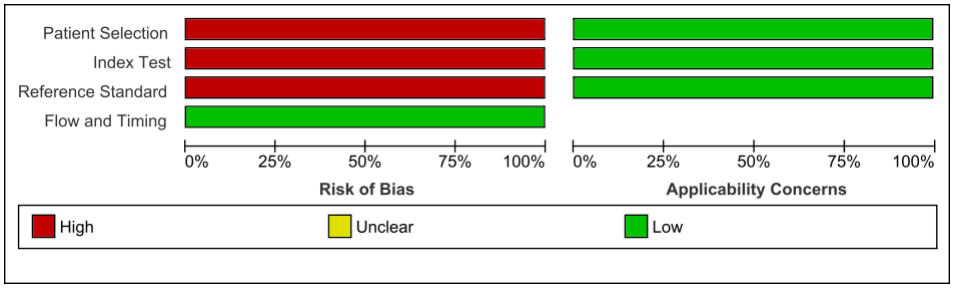

Figure 3. Quality of included studies according to QUADAS-2 guidelines

Differentially expressed miRNAs from ranking

A total of 106 differentially expressed miRNAs were identified of which 19 were reported in at least two studies. Out of these 106 miRNAs, 55 were upregulated (**Table 2**). The most frequently found up-regulated miRNA was miR-205, which was reported in 10 articles and showed a mean fold change of 198.08 when tested in 134 EC patients and 64 control patients. Furthermore miR-200c was reported in 8 articles to be up-regulated in EC (mean fold change 27.99), in addition the following miRNAs were reported in 5 articles: miR-223 (mean fold change 40.17), miR-182 (mean fold change 11.41), miR-183 (mean fold change 8.75) and miR-200a (mean fold change 5.20).

Table 2. Up-regulated miRNAs reported in all studies in all specimen types with fold changes

MiRNA	Number of studies	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
miR-205	10	134	64	198.08	4
miR-200c	8	164	83	27.99	4
miR-223	5	144	99	40.17	3
miR-182	5	141	79	11.41	3
miR-183	5	141	79	8.75	2
miR-200a	5	155	89	5.20	4
miR-135b	3	96	35	35.59	2
miR-429	3	117	61	6.83	3
miR-141	3	103	51	4.25	2
miR-200b	3	103	51	3.86	2
miR-200a*	2	73	31	26.96	1
miR-222	2	26	22	19.16	1
miR-141-3p	2	77	68	15.36	2
miR-200c-3p	2	28	62	15.34	1
miR-186	2	26	22	11.39	1
miR-200b*	2	73	31	6.52	1
miR-15b	2	40	49	6.10	1
miR-106a	2	68	48	2.80	2
miR-135a	1	23	4	34.05	1
miR-205-5p	1	28	62	24.19	1
miR-182-5p	1	28	62	22.76	1
miR-200b-3p	1	28	62	16.19	1
miR-92a	1	73	31	15.63	1
miR-9-5p	1	16	34	15.05	1
miR-27a	1	40	49	5.63	1
miR-210	1	38	28	5.23	1
miR-96	1	73	31	4.27	1
miR-194	1	38	28	4.11	1
miR-95	1	38	28	4.09	1
miR-155	1	38	28	3.87	1
miR-18a*	1	30	20	3.65	1
miR-222-3p	1	12	28	3.43	1
miR-96-5p	1	49	6	3.20	1
miR-103	1	38	28	3.00	1
miR-151	1	38	28	2.85	1
miR-34a	1	30	20	2.63	1
miR-92a-1*	1	30	20	2.47	1
miR-887-5p	1	50	50	2.41	1
miR-20a*	1	30	20	2.34	1
miR-106b*	1	30	20	2.34	1
miR-449a	1	73	31	2.26	1
miR-17*	1	30	20	1.99	1
miR-185	1	30	20	1.85	1
miR-1228	1	73	31	1.18	1
miR-146	1	141	20	NR	0
miR-425	1	141	20	NR	0
miR-1290	1	73	31	NR	0
miR-944	1	68	20	NR	0
miR-16	1	67	15	NR	0
miR-128a	1	67	15	NR	0

table continues

MiRNA	Number of studies	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
miR-148b	1	67	15	NR	0
miR-196a	1	67	15	NR	0
miR-301	1	67	15	NR	0
miR-582-5p	1	67	15	NR	0
miR-499	1	28	14	NR	0

NR = not reported
*** mean fold change as found by qRT-PCR*

Other promising up-regulated miRNAs listed in 3 articles were: miR-135b, miR-429, miR-141 and miR-200b. MiRNAs only listed up-regulated in one or two articles can be found in **Table 2**.

There was less consensus on miRNAs that are down-regulated in women with EC (**Table 3**), with 44 different miRNAs being down-regulated. However, all miRNAs were only reported in 1 article[15,26,35,37,39–44]. MiR-137 and miR-129-3p showed the largest mean fold change (115.15 and 42.30 respectively) but were only found in a small cohort (sample size EC 23 and sample size control 4 patients)[38]. MiR-410 was found to have a mean fold change of 13.91 and was tested in a slightly larger cohort (sample size EC 73 and sample size control 31 patients)[40].

Table 3. Down-regulated miRNAs reported in all studies in all specimen types with fold changes

MiRNA	Number of studies	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
miR-137	1	23	4	115.15	1
miR-129-3p	1	23	4	42.30	1
miR-410	1	73	31	13.91	1
miR-503	1	14	10	8.60	1
miR-1247	1	30	20	5.31	1
miR-376c	1	30	20	3.64	1
miR-377	1	30	20	3.34	1
miR-26a-5p	1	49	6	3.10	1
miR-214	1	30	20	2.90	1
miR-150-5p	1	49	6	2.70	1
miR-370	1	30	20	2.68	1
let-7f-5p	1	49	6	2.60	1
miR-26b-5p	1	49	6	2.60	1
let-7c-5p	1	49	6	2.50	1
miR-23b-3p	1	49	6	2.40	1
miR-125b-5p	1	49	6	2.30	1
miR-126-3p	1	49	6	2.30	1
miR-195-5p	1	49	6	2.20	1
miR-424-5p	1	49	6	2.20	1
miR-374a-5p	1	49	6	2.10	1
let-7a-5p	1	49	6	2.00	1
let-7e-5p	1	49	6	2.00	1

table continues

MiRNA	Number of studies	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
miR-125a-5p	1	49	6	2.00	1
miR-542-5p	1	14	10	2.00	1
miR-337-5p	1	30	20	1.94	1
miR-1305	1	73	31	1.77	1
miR-758	1	30	20	1.61	1
miR-300	1	30	20	1.56	1
miR-93	1	176	100	NR	0
miR-125	1	67	15	NR	0
miR-34	1	67	15	NR	0
miR-30a-3p	1	40	26	NR	0
miR-301b	1	34	14	NR	0
miR-10b	1	28	14	NR	0
miR-195	1	28	14	NR	0
miR-30a-5p	1	28	14	NR	0
miR-101	1	21	7	NR	0
miR-10b*	1	21	7	NR	0
miR-133a	1	21	7	NR	0
miR-133b	1	21	7	NR	0
miR-152	1	21	7	NR	1
miR-29b	1	21	7	NR	0
miR-34b	1	21	7	NR	0
miR-411	1	21	7	NR	0

NR = not reported
*** mean fold change as found by qRT-PCR*

For 7 miRNAs (miR-203, miR-21, miR-204, miR-9, miR-199b, miR-99a and miR-100) an inconsistent altered expression was found (**Table 4**). MiR-203 was found up-regulated in 3 studies with mean fold change 4.19, however was also found down-regulated in one study[28,40,45,46].

Table 4. MiRNAs with inconsistent direction of change

MiRNA	Number of studies	Up/down-regulation	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
miR-203	3	Up	111	59	4.19	2
	1	Down	60	10	0.073	1
miR-21	1	Up	67	15	NR	0
	1	Down	40	26	NR	0
miR-204	1	Up	26	22	5.79	1
	1	Down	46	28	NR	0
miR-9	1	Up	73	31	5.46	1
	1	Down	34	14	NR	0
miR-199b	1	Up	4	14	2.89	1
	1	Down	73	31	3.52	1
miR-99a	1	Up	4	14	1.96	1
	1	Down	73	31	3.29	1
miR-100	1	Up	4	14	1.65	1
	1	Down	73	31	2.56	1

NR = not reported
*** mean fold change as found by qRT-PCR*

Differentially expressed miRNAs per specimen type

The included articles were subdivided according to specimen subtype: tissue specimens (19 articles), serum/plasma (8 articles), urine (1 article) and LBC (1 article) (**Supplementary Tables 5-12**). For tissue specimens, the results were similar to the previously reported results with the only difference being that miR-200c was listed in 7 articles (previously 8) and miR-223 in 2 (previously 5). For serum/plasma samples miR-223 was most often reported up-regulated in 3 studies, followed by miR-222, miR-186 and miR-203 in 2 studies. MiR-205 was not reported in serum/plasma samples to be deregulated.

Differentially expressed miRNAs per subtype EC

Furthermore, the articles were subdivided according to EC subtype: looking at endometrioid versus serous carcinomas, to distinguish if a miRNA signature can be found per subtype. There were 12 studies looking at EEC only[29,30,40–43,45,47–50] and one study by Devor et al. who reported on both endometrioid and serous carcinomas but reported the subtypes separately[38]. There was only one other study looking at serous carcinoma only[37]. For EEC, data was in line with previously reported data; the most often up-regulated miRNA was miR-205 (cited in 7 articles), followed by miR-200c (6 articles), miR-182, and -200a (5 articles), miR-183 (4 articles) and miR-135b, -429, -141 and -223 (3 articles), data not shown. For serous type endometrial carcinoma, miR-205 was found up-regulated in both studies, furthermore miR-200c, -135a and -135b were up-regulated in one of the two studies.

Discussion

EC is the most common malignancy of the female genital tract in developed countries with rising incidence and mortality rates[51]. Although EC is generally associated with good prognosis, patients presenting with advanced or recurrent EC have poor survival rates[6]. MiRNAs have been shown to play a significant role in tumour genesis and progression and therefore warrant a clinical potential as diagnostic and prognostic marker in EC. In this review a systematic search was conducted to identify the feasibility and overall diagnostic value of miRNA expression in EC.

MiR-205 was most consistently found to be up-regulated, with a differential expression reported among ten studies and mean fold change of 198.08[33,36–38,40,41,45,47,49,50]. MiR-205 is involved in the regulation of PTEN expression in EC and leads to reduced cell apoptosis[52]. Furthermore, miR-205 represses the tumour suppressor gene JPH4, promoting tumorigenesis and tumour progression[52]. However, miR-205 is not only up-regulated in EC, but also in other cancer sites such as lung and ovarian cancer[53,54]. Therefore, miR-205 on its own seems not

adequate as a diagnostic test for the detection of EC. Lee et al. found a panel of six miRNAs (miR-205, miR-200a, miR-200c, miR-182, miR-183 and miR-21) to have an area under the curve (AUC) of 0.961, sensitivity and specificity of 91% and 94% respectively in discriminating EC from hyperplasia or normal tissue[47]. The results of this systematic review confirm the importance of these miRNAs in endometrial carcinogenesis.

MiR-200c and miR-200a were reported consistently up-regulated in 8 and 5 studies respectively, in addition miR-200b, miR-429 and miR-141 were reported up-regulated in 3 studies. These miRNAs are part of the miR-200 family, the expression and function of which has been well documented in various tissues and has been suggested to play an important role in inhibiting cell malignant transformation and preventing tumour initiation[55]. The miR-200 family targets the expression of many genes, including ZEBs (Zinc finger E-box-binding homeobox), which are the transcription factors that regulate cellular transformation, more specifically epithelial-to-mesenchymal transition (EMT), during cancer development and progression through repression of adhesion molecules such as E-cadherin[56]. Members of the miR-200 family also host diagnostic and prognostic potential in other cancer sites such as gastric, ovarian, lung and colorectal cancer[57–60].

Furthermore, miR-182, miR-183 and miR-223 were found up-regulated in 5 articles and miR-135b was found to be up-regulated in 3 articles. MiR-182 promotes cell proliferation by targeting the tumour suppressor gene TCEAL7, miR-183 targets CPEB1 while miR-223 targets IGF-1R[61–63]. MiR-135b targets FOXO1 to promote cell proliferation in EC cells[64]. These miRNAs are also up-regulated in patients with non-small cell lung cancer, colorectal-, prostate- and pancreatic cancer[65–67]. In addition, a recent systematic review by Delangle et al. found miR-182 and miR-183 to be associated with poorer prognosis in terms of overall survival and recurrence-free survival in EC[68]. They therefore conclude that miRNA analysis merits a role as a prognostic factor in the management of patients with EC. For other gynaecological cancer sites such as ovarian and cervical cancer, the diagnostic and prognostic significance of different panels of miRNAs have been investigated and also show promising results[19,69].

The distinct panel identified in this systematic review (miR-205, the miR-200 family, miR-135b, -182, -183, and -223) is promising in the detection of EC. However, some of the same miRNAs are also upregulated in colorectal cancer, therefore, we suggest that these miRNAs may be used in the diagnosis of women presenting with specific symptoms such as abnormal or postmenopausal bleeding. Since miRNAs can be detected in a huge variety of bodily fluids including urine and since miRNAs are stable in urine, urine seems like a promising non-invasive test for the detection of EC[70]. Urinary miRNAs have shown potential in the detection

of bladder- and prostate cancer[71,72], however in EC only one study has used urine for the detection of miRNA[31]. In addition, Zavesky et al. compared urinary miRNA expression levels of pre- and post-surgery ovarian cancer samples and between patients with ovarian and endometrial cancer (n = 10) and healthy controls and proposed urinary miRNA should be further investigated to test the diagnostic potential of urine miRNAs in gynaecological cancers[73]. A urinary diagnostic test will potentially allow for easier access to care, help reduce anxiety among women and could prevent the need for painful biopsies. Another potential is possibly reducing the burden of travelling long distances to the hospital and costs for patients. In addition, if fewer patients need to be referred to the hospitals this will have a potential cost reduction implication. Further studies should therefore determine if urinary miRNA detection is a valid non-invasive way of reliably detecting EC.

In addition to the need for new biomarkers to detect EC, there is also the need for these biomarkers to accurately distinguish between low (grade 1 and grade 2) or high-grade (grade 3) EEC[74]. Ratner et al. reported unique miRNA signatures for EEC, serous carcinomas and uterine carcinosarcomas, but no difference between grade 1 and grade 3 EEC[75]. Further research may help determine if miRNA can accurately distinguish low-grade EEC from high-grade EEC.

Furthermore, there is an increasing interest in improving the preoperative classification of EC, in order to allow for non-invasive and more precise diagnostic options for patients. In 2013, the Cancer Genome Atlas proposed an additional division of EC into four molecular subtypes: Polymerase-ε (POLE) ultramutated, microsatellite instability (MSI) hypermutated, copy-number (CN) low and CN high[76]. CN low include most endometrioid tumours and are frequently associated with mutations in PTEN, CTNNB1, PIK3CA, ARID1A and KRAS, whereas CN high include serous tumours and 25% of high-grade endometroid tumours[77]. POLE and MSI mutated EC tumours show better survival outcomes[76]. Since miR-205 is involved in the regulation of PTEN expression in EC, miRNA detection could potentially be of use in a molecular based classification system for correct preoperative diagnosis and classification of EC.

Although these findings are encouraging, the main limitations to the usage of miRNAs include different platforms of miRNA profiling, including microarray, next generation sequencing and RT-qPCR, which leads to inconsistency and difficulties in comparing results[78]. It is difficult to compare data gained with different miRNA profiling platforms, as they are only somewhat reproducible and even intraplatform variation is common[79]. Due to differences in the accuracy, reproducibility, sensitivity and specificity of PCR kits, the reproducibility of miRNA detection and quantification is relatively low[80]. Furthermore, most miRNAs are not cancer type specific, therefore an EC specific miRNA signature needs further testing to determine

if these miRNAs can accurately distinguish EC from benign tissue. This systematic review has shown the most promising miRNAs to be miR-205, -200a, -200b, -200c, -141, -429, -135b, -182, -183 and -223 and therefore need further testing.

The strengths of this study include a comprehensive systematic search performed by two reviewers independently. In addition, to improve comparability, we have only included studies in this review that used RT-qPCR for miRNA detection. Furthermore, all included studies varied in the subtype EC which they assessed; some studies included only EEC, others only NEEC and some studies combined the two subtypes in their analyses, even though EEC and NEEC vary in pathophysiology and prognosis. However, when subdividing the articles into endometrioid versus serous endometrial carcinoma, the data found seems in line with data found when combining subtypes.

The limitations to this systematic review are the following; there is a high heterogeneity in methodologies used (different platforms, analysis software and normalisation strategies) and specimen samples (FFPE, fresh frozen tissue, serum, plasma, urine, LBC) among the different studies included. When subdividing the articles per specimen type, we found a different expression for tissue specimens compared to serum/plasma samples. In addition, the majority of studies only included small sample sizes. Finally, the majority of studies were conducted in Asia and no studies were conducted in Africa, South-America or Oceania.

Conclusion

This systematic review shows that miRNAs are potential promising biomarkers for the diagnosis of EC, however no sufficient high-quality evidence is available to draw hard conclusions. The combination of miR-205, the miR-200 family, miR-135b, -182, -183 and -223 needs further testing in larger studies with standardised protocols to improve the accuracy of using these miRNAs in diagnosing EC in the future. In addition, the potential of urine as a non-invasive biofluid should be investigated.

Clinical significance

MiRNA can potentially be used in low resource settings where there is lack of trained histopathologists. In addition, a urinary miRNA test can potentially be used as a non-invasive test for the detection of EC. This will allow for easier access to care and reduction of travelling long distances to the hospital for patients. It could mean a cost reduction for the hospital, if patients can be seen in the community instead of hospitals.

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Supplemental material

Supplementary Table 5. Up-regulated miRNAs in tissue samples

MiRNA	Number of studies	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
miR-205	10	134	63	198.08	4
miR-200c	7	126	55	27.99	4
miR-182	5	141	79	11.41	3
miR-183	5	141	79	8.75	2
miR-200a	5	121	75	5.20	4
miR-135b	3	96	35	35.59	2
miR-429	3	117	61	6.83	3
miR-200b	3	103	51	3.86	2
miR-200a*	2	73	31	26.96	1
miR-141-3p	2	65	30	22.95	2
miR-200b*	2	73	31	6.52	1
miR-223	2	38	28	4.9	1
miR-141	2	30	20	4.70	1
miR-106a	2	68	48	2.80	2
miR-135a	1	23	4	34.05	1
miR-205-5p	1	16	24	31.52	1
miR-200b-3p	1	16	24	27.68	1
miR-182-5p	1	16	24	27.30	1
miR-200c-3p	1	16	24	24.40	1
miR-92a	1	39	17	15.63	1
miR-9-5p	1	16	34	15.05	1
miR-9	1	73	31	5.46	1
miR-210	1	38	28	5.23	1
miR-96	1	73	31	4.27	1
miR-194	1	38	28	4.11	1
miR-95	1	38	28	4.09	1
miR-155	1	38	28	3.87	1
miR-18a*	1	30	20	3.65	1
miR-96-5p	1	49	6	3.20	1
miR-103	1	38	28	3.00	1
miR-151	1	38	28	2.85	1
miR-34a	1	30	20	2.63	1
miR-92a-1*	1	30	20	2.47	1
miR-20a*	1	30	20	2.34	1
miR-106b*	1	30	20	2.34	1
miR-17*	1	30	20	1.99	1
miR-185	1	30	20	1.85	1
miR-222-3p	1	16	24	1.80	1
miR-146	1	141	20	NR	0
miR-425	1	141	20	NR	0
miR-944	1	68	20	NR	0
miR-15b	1	67	15	NR	0
miR-16	1	67	15	NR	0
miR-128a	1	67	15	NR	0
miR-148b	1	67	15	NR	0
miR-196a	1	67	15	NR	0
miR-301	1	67	15	NR	0
miR-582-5p	1	67	15	NR	0
miR-499	1	28	14	NR	0

NR = not reported

*** mean fold change as found by qRT-PCR*

Supplementary Table 6. Up-regulated miRNAs in serum/plasma samples

MiRNA	Number of studies	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
miR-223	3	66	71	57.81	2
miR-222	2	26	22	19.16	1
miR-186	2	26	22	11.39	1
miR-203	2	79	44	NR	0
miR-15b	1	40	49	6.10	1
miR-204	1	26	22	5.79	1
miR-27a	1	40	49	5.63	1
miR-199b	1	34	14	2.89	1
miR-887-5p	1	50	50	2.41	1
miR-99a	1	34	14	1.96	1
miR-100	1	34	14	1.65	1
miR-200a	1	34	14	NR	0
miR-141	1	34	14	NR	0
miR-92a	1	34	14	NR	0
miR-449a	1	34	14	NR	0
miR-1228	1	34	14	NR	0
miR-1290	1	34	14	NR	0
miR-205	1	12	12	NR	0
miR-135b	1	12	12	NR	0

NR = not reported
*** mean fold change as found by qRT-PCR*

Supplementary Table 7. Up-regulated miRNAs in urine samples

MiRNA	Number of studies	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
miR-200c-3p	1	22	5	NR	0

NR = not reported
*** mean fold change as found by qRT-PCR*

Supplementary Table 8. Up-regulated miRNAs in LBC samples

MiRNA	Number of studies	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
miR-182-5p	1	12	28	18.21	1
miR-205-5p	1	12	28	16.85	1
miR-141-3p	1	12	28	11.45	1
miR-200c-3p	1	12	28	6.27	1
miR-200b-3p	1	12	28	4.69	1
miR-222-3p	1	12	28	3.43	1

NR = not reported
*** mean fold change as found by qRT-PCR*

Supplementary Table 9. Down-regulated miRNAs in tissue samples

MiRNA	Number of studies	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
miR-137	1	23	4	115.15	1
miR-129-3p	1	23	4	42.30	1
miR-410	1	73	31	13.91	1
miR-503	1	14	10	8.60	1
miR-1247	1	30	20	5.31	1
miR-376c	1	30	20	3.64	1
miR-199b	1	73	31	3.52	1
miR-377	1	30	20	3.34	1
miR-99a	1	73	31	3.29	1
miR-26a-5p	1	49	6	3.10	1
miR-214	1	30	20	2.90	1
miR-150-5p	1	49	6	2.70	1
miR-370	1	30	20	2.68	1
let-7f-5p	1	49	6	2.60	1
miR-26b-5p	1	49	6	2.60	1
miR-100	1	73	31	2.56	1
let-7c-5p	1	49	6	2.50	1
miR-23b-3p	1	49	6	2.40	1
miR-125b-5p	1	49	6	2.30	1
miR-126-3p	1	49	6	2.30	1
miR-195-5p	1	49	6	2.20	1
miR-424-5p	1	49	6	2.20	1
miR-374a-5p	1	49	6	2.10	1
let-7a-5p	1	49	6	2.00	1
let-7e-5p	1	49	6	2.00	1
miR-125a-5p	1	49	6	2.00	1
miR-542-5p	1	14	10	2.00	1
miR-337-5p	1	30	20	1.94	1
miR-1305	1	73	31	1.77	1
miR-758	1	30	20	1.61	1
miR-300	1	30	20	1.56	1
miR-125	1	67	15	NR	0
miR-34	1	67	15	NR	0
miR-30a-3p	1	28	14	NR	0
miR-10b	1	28	14	NR	0
miR-195	1	28	14	NR	0
miR-30a-5p	1	28	14	NR	0
miR-101	1	21	7	NR	0
miR-10b*	1	21	7	NR	0
miR-133a	1	21	7	NR	0
miR-133b	1	21	7	NR	0
miR-152	1	21	7	NR	1
miR-29b	1	21	7	NR	0
miR-34b	1	21	7	NR	0
miR-411	1	21	7	NR	0

NR = not reported

*** mean fold change as found by qRT-PCR*

Supplementary Table 10. Down-regulated miRNAs in serum/plasma samples

MiRNA	Number of studies	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
miR-93	1	176	100	NR	0
miR-204	1	46	28	NR	0
miR-30a-3p	1	40	26	NR	0
miR-9	1	34	14	NR	0
miR-301b	1	34	14	NR	0
miR-21	1	12	12	NR	0

NR = not reported
*** mean fold change as found by qRT-PCR*

Supplementary Table 11. MiRNAs with inconsistent direction of change in tissue

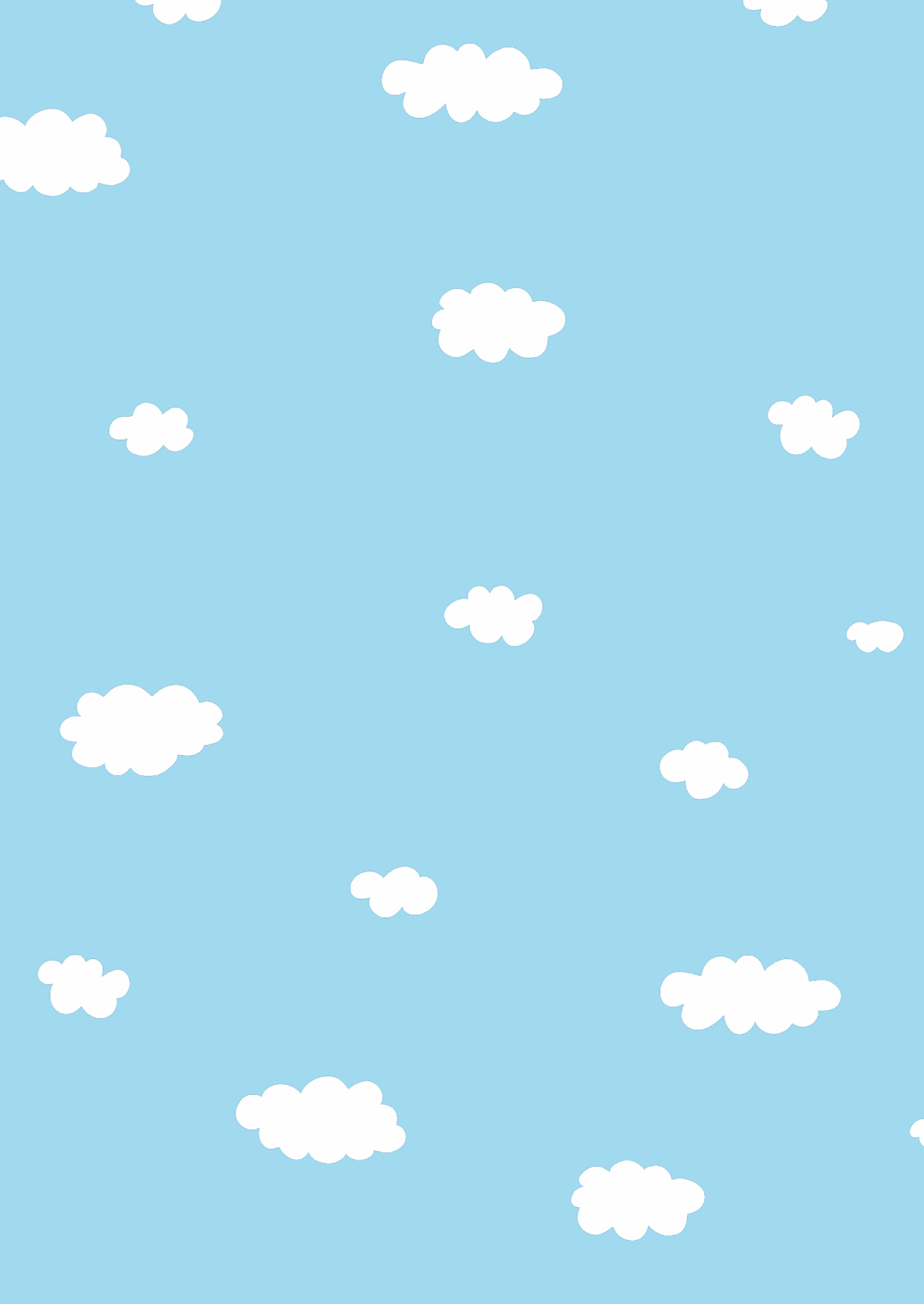
MiRNA	Number of studies	Up/down-regulation	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
miR-203	2	Up	111	59	4.19	2
	1	Down	60	10	0.073	1
miR-21	1	Up	67	15	NR	0
	1	Down	28	14	NR	0

NR = not reported
*** mean fold change as found by qRT-PCR*

Supplementary Table 12. MiRNAs with inconsistent direction of change in plasma/serum

MiRNA	Number of studies	Up/down-regulation	Sample size EC	Sample size control	Mean fold change**	Studies with fold change reported
MiR-204	1	Up	26	22	5.79	1
	1	Down	46	28	NR	0

NR = not reported
***mean fold change as found by qRT-PCR*



Chapter 8

Analysing microRNA for the diagnosis of endometrial cancer: A step in the right direction?

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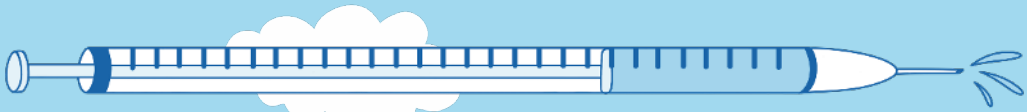
Submitted



Abstract

Introduction

MicroRNAs (miRNAs) are noncoding RNAs that regulate gene expression and contribute to the development of cancer. MiRNAs have been shown to be stable in urine, serum and tissue samples. They may be promising biomarkers for non-invasive detection of endometrial cancer (EC).

Methods

A retrospective cohort study of women diagnosed with EC between January 2017 and December 2017 was performed at the Royal Cornwall Hospital. Archived formalin-fixed paraffin-embedded samples were obtained from patients with EC and healthy female subjects. MiRNA was isolated and qPCR was used to detect expression levels of miRNAs.

Results

A total of 76 women were included; 36 EC patients, 40 healthy controls. A distinct panel of miR-200a, miR-200b, miR-200c, miR-205 and miR-182 showed AUC of 0.958, sensitivity 92%, specificity 89%, positive predictive value of 89% (95% confidence interval (CI) 82 – 94%) and negative predictive value of 91% (95% CI 85 – 96%) in diagnosing EC. High miR-182 expression levels were significantly related to high-grade endometrioid tumours compared to low-grade.

Conclusion

We demonstrated high diagnostic accuracy of miRNA for detecting EC. In addition, miRNA contributed to improved distinguishing between high-grade and low-grade endometrioid tumours. Validation of miRNA expression levels in urine will be performed in order to further optimise a non-invasive diagnostic tool.

Introduction

Endometrial cancer (EC) is the 6th most commonly occurring cancer in women and the 15th most commonly occurring cancer overall[1]. Since 1990, the incidence of EC has increased by 57% in the United Kingdom (UK), which is attributed to the rise in obesity[2]. EC is generally diagnosed early since patients present early with bleeding abnormalities and consequently overall good prognosis is seen[3]. Diagnosis is achieved by combination of transvaginal ultrasound scan and histological confirmation on endometrial biopsy. Outpatient endometrial sampling with pipelle is more cost-effective than immediate hysteroscopy, however high numbers of technical problems (12-23%) and insufficient amount of tissue (16-68%) have been described[4]. Furthermore, endometrial sampling is invasive and can be uncomfortable, with Visual Analogue Scale pain score of 6.5 in postmenopausal women[5]. Even with sufficient amount of tissue, there can be large discordancy between pre- and postoperative tumour classification[6]. Discordances in grading and histologic subtype in preoperative and final diagnosis can lead to either undertreatment or overtreatment with unnecessary surgical procedures and associated complications. Therefore, the identification of validated and non-invasive diagnostic biomarkers are needed to accurately diagnose EC and aid correct preoperative classification.

MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression which play crucial roles in biological processes including cellular differentiation, proliferation and apoptosis[7]. Abnormal expression of miRNAs can contribute to the development of cancer and can therefore be a candidate in either detection or monitoring cancer treatment[8]. A recent systematic review showed a promising diagnostic potential of miRNA panel for EC[9]. MiR-205, the miR-200 family (consisting of miR-200a, -200b, -200c, -141 and -429) and miR-135b, -182, -183 and -223 were found up-regulated most consistently[9].

MiRNA has been shown to be stable in serum and urine samples as well as formalin-fixed paraffin-embedded (FFPE) tissue samples, which are routinely archived in most hospitals and are therefore widely used for the discovery of clinically useful biomarkers[10]. Therefore, in this study we aim to test and validate the potential of a distinct miRNA panel in FFPE tissue as a first step in optimising it as diagnostic tool for the detection of EC.

Materials and methods

Study design and participants

This is a retrospective cohort study in the Royal Cornwall Hospital, Truro, UK, in collaboration with Department of Obstetrics and Gynecology, University

Medical Centre Freiburg, Germany, on women diagnosed with primary EC. Samples were obtained from archived uterine FFPE samples from consecutive women who underwent surgical treatment (hysterectomy with bilateral salpingo-oophorectomy) between January 2017 and December 2017 in the Royal Cornwall Hospital. In total, 10 women were excluded for following reasons: histological diagnosis of complex atypical hyperplasia (n = 5), carcinoma in situ (n = 1), micro-invasive cervical squamous carcinoma (n = 1), mucinous borderline ovarian tumour (n = 1), round cell sarcoma (n = 1) or poorly preserved endometrium (n = 1). The total study population consisted of 80 women; 40 consecutive FFPE samples from women who underwent hysterectomy for EC and 40 who had hysterectomy for benign conditions that served as control group.

MiRNA expression profiles

A total of 43 miRNAs have previously been reported deregulated in EC, therefore those miRNAs were investigated in this study[9]. Using findings from a recent systematic review, we furthermore mainly focused on miR-205, -200a, -200b, -200c, -223, -182, -183, -135b, -429, and miR-141 as they were previously shown to be most consistently up-regulated[9].

MiRNA isolation and quantitative real-time PCR (qPCR) were performed in the Department of Obstetrics and Gynecology in Freiburg.

Sample processing and miRNA isolation

Deparaffinisation of the FFPE tissue was achieved by soaking the samples twice in 1 ml xylene at 60°C in a thermomixer (Eppendorf, Hamburg, Germany) at 1,000 revolutions per minute (rpm), then spin at 13,000 rpm. Removal of xylene was achieved by washing the samples three times with 1 ml 100% ethanol, then spinned at 13,000 rpm. The sections were dried at 60°C for 10 minutes. Tissue homogenisation was attained by precellys bead mill at 6,100 rpm for 20 seconds (Bertin technologies; Peqlab Erlangen, Germany). Isolation and purification of the miRNA was utilized by EURx® FFPE RNA purification kit according to the manufacturer's protocol. The RNA concentration was determined using IMPLEN N60 Nanophotometer (München, Germany). RNA was stored at -20˚C until further processing.

Reverse transcription (RT)

For cDNA generation poly(A) tailing-based RT was performed, using a total reaction volume of 20 µl. The RT reaction mix included: 4 µl RT-buffer (5X), 1 µl 5 µM poly A adapter/primer (Integrated DNA Technologies, Inc.), 1 µl 5 mM dNTPs (Jena Bioscience), 0.25 µl Maxima™ H Minus reverse transcriptase (Thermo Fisher Scientific, Inc.), 0.25 µl SUPERase In™ RNase inhibitor (Thermo Fisher Scientific, Inc.), 0.5 µl 10 mM ATP (New England BioLabs, Inc.), 0.25 µl poly A polymerase

(New England BioLabs, Inc.) and 100 ng total RNA. cDNA samples were diluted in H_2O to a final volume of 200 µl and stored at -20°C until further processing.

Quantitative real-time PCR (qPCR) of miRNAs

Quantitative determination of miRNA expression levels was implemented by real-time qPCR on LightCycler® 480 (Roche, Mannheim, Germany). The PCR reaction set up contained: 1 µl cDNA, in-house qPCR buffer (containing TRIS pH8.1, dATP, dCTP, dGTP, dTTP, magnesium, potassium ammonium, SYBRGreen (Jena Bioscience, Jena, Germany), enhancers) 0.25 units HotStart Taq Polymerase (Jena Bioscience) in a total volume of 10 µl. QPCR primers were designed by means of 'miRprimer' software. The used qPCR program contained the following steps: initial denaturation (95°C; 2 minutes); 40 cycles of denaturation (95°C; 5 seconds) / annealing/extension (60°C; 30 seconds) and a final melting curve analysis step. Based on DC_t method relative quantification of miRNA expression levels was conducted in a duplicate analysis normalized on the geometric mean of the endogenous controls RNU-44 RNU-48 which were identified as stably expressed between malignant and normal tissues[11].

Statistical analysis

For normal distributed data continuous outcomes were presented as medians and interquartile range, categorical data were presented as frequencies and proportions. The non-parametric Mann-Whitney U Test was used for the comparison between EC and benign controls and the Fisher Exact Test for the comparison between high-grade and low-grade endometrioid EC. The Benjamini-Hochberg correction was applied to adjust for multiple comparisons with critical value for false discovery rate of 0.25[12]. The receiver-operator characteristics (ROC) curve was constructed, and the cut-off values of each miRNA were selected. The sensitivity and specificity in differentiating EC from benign endometrium were calculated for each cut-off and the area under the curve (AUC) was estimated. A stepwise logistic regression was used to establish a model as a surrogate marker to diagnose EC. P-values less than 0.05 were considered significant for all tests. Data were analysed with IBM SPSS statistics version 25.0.

Ethical approval

This study was approved by the Health Research Authority (HRA) and Health and Care Research Wales (HCRW) with REC reference number 19/ES/0007.

Results

Demographics

Of the 80 women included in this study, FFPE samples of 4 women showed insufficient miRNA extraction and were therefore excluded for analysis. The

demographics of the total study population of 76 women are presented in **Table 1**. The majority of EC patients had endometrioid histology (78%), followed by serous (11%). Most patients were diagnosed with FIGO stage 1 (58%) and grade 1 (36%).

Table 1. Patient characteristics

Variable	Value
Number of women	76
Histology (%)	
Benign	40 (53)
Endometrioid	28 (37)
Serous	4 (5)
Carcinosarcoma	2 (3)
Clear cell	1 (1)
Mucinous	1 (1)
Ethnicity (%)	
Caucasian	73 (96)
Any other ethnic group	2 (3)
Unknown	1 (1)
Parity (%)	
0	15 (20)
≥1	59 (78)
Unknown	2 (3)
FIGO stage (%)	
1	21 (58)
2	4 (11)
3	6 (17)
4	2 (6)
Unknown	3 (8)
Grade (%)	
1	13 (36)
2	12 (33)
3	9 (25)
Unknown	4 (11)

Abbreviations: FIGO = International Federation of Gynecology and Obstetrics

For the control group, patients underwent hysterectomy due to menorrhagia (n = 32), ovarian cysts (n = 3), endometriosis (n = 2), pelvic pain (n = 2) or recurrent abnormal Pap smears (n = 1). Histology showed proliferative endometrium (n = 21), secretory phase endometrium (n = 15) or atrophic endometrium (n = 4) and adenomyosis in 12 patients.

EC patients were significantly older with median age of 68 years compared to 45 years for the control group ($p < 0.001$, **Table 2**), and had higher Body Mass Index (BMI), median of 33 m/kg^2 compared to 29 m/kg^2 in the control group ($p = 0.009$). There was no difference in total amount of comorbidities; however, diabetes mellitus was more frequently seen in EC patients compared to healthy controls ($p = 0.008$).

Table 2. Differences in baseline characteristics

Characteristics	Benign (N = 40)	Endometrial cancer (N = 36)	p-value
Age, median (years) (IQR)	45 (7)	68 (17)	**<0.001***
BMI, median (m/kg^2) (IQR)	29 (8)	33 (6)	**0.009***
Comorbidities (%)			
None	12 (30)	6 (17)	0.28
One	14 (35)	11 (31)	
Two or more	14 (35)	18 (50)	
Unknown	0 (0)	1 (3)	
Diabetes Mellitus (%)			
Yes	1 (2)	8 (22)	**0.008***
No	39 (98)	28 (78)	
ECOG (%)			
0	40 (100)	21 (58)	**0.001***
1	0 (0)	8 (22)	
2	0 (0)	2 (6)	
3	0 (0)	1 (3)	
Unknown	0 (0)	4 (11)	
Smoking status (%)			
Non-smoker	23 (58)	28 (78)	**0.026***
Smoker	12 (30)	2 (6)	
Ex-smoker	5 (12)	4 (11)	
Unknown	0 (0)	2 (6)	

Abbreviations: BMI = Body Mass Index, IQR = Interquartile Range, ECOG = Eastern Cooperative Oncology Group

MiRNA expression profiles

Of the 43 miRNAs investigated, 27 miRNAs demonstrated significantly different altered expression patterns (**Table 3**), which remained significant after correction for multiple testing. See **Supplementary Table 1** for the median expression levels of miR-205, -200a, -200b, -200c, -223, -182, -183, -135b, -429, and miR-141.

MiRNA panel

The 10 most promising miRNAs in diagnosing EC in previous literature were analysed for their diagnostic value by construction of ROC curves to estimate the sensitivity and specificity of these miRNAs. The individual miRNAs exhibited AUC values of 0.603 – 0.868 (**Supplementary Figure 1** and **Table 4**) in distinguishing EC from benign endometrium, revealing 62-100% sensitivity and 21-88% specificity. To further determine the diagnostic value of a panel of miRNAs, a stepwise logistic regression analysis was applied to the 10 miRNAs. The best fitting model was a panel of miR-200a, miR-200b, miR-200c, miR-205 and miR-182 with AUC of 0.958 (Standard Error 0.021, **Figure 1**). Given a specificity of 89%, the composite panel revealed a sensitivity of 92% in differentiating EC from normal endometrium and a positive predictive value of 89% (95% Confidence Interval (CI) 82% - 94%) and negative predictive value of 91% (95% CI 85% – 96%). **Supplementary Figure 2** shows the boxplots of expression levels of these five miRNAs.

Table 3. Up/downregulation pattern and expression values for all tested miRNAs

MiRNA	Benign, median (range)	Cancer, median (range)	p-value
Down-regulated			
let-7a	*2.18 (7.17)*	*0.99 (4.87)*	**0.021***
miR-10b	0.40 (1.35)	0.30 (1.56)	0.06
miR-15a	0.04 (0.91)	0.03 (0.16)	0.99
miR-16	0.06 (13.87)	0.06 (2.14)	0.69
miR-20b	0.01 (0.10)	0.01 (0.03)	0.90
miR-100	0.73 (3.79)	0.54 (4.55)	0.11
miR-125b	*2.84 (14.37)*	*1.92 (12.84)*	**0.039***
miR-134	*0.00 (0.12)*	*0.00 (0.07)*	**0.028***
miR-195	*0.34 (6.83)*	*0.13 (6.23)*	**<0.001***
miR-222	*0.10 (1.53)*	*0.06 (3.35)*	**0.010***
miR-574-3p	*0.07 (0.19)*	*0.03 (0.09)*	**<0.001***
let-7i	0.29 (0.95)	0.22 (0.79)	0.16
miR-26b	0.65 (3.05)	0.44 (1.35)	0.11
miR-107	*0.14 (0.95)*	*0.11 (0.31)*	**0.006***
miR-423	0.10 (0.95)	0.07 (0.26)	0.08
miR-424	*0.23 (2.07)*	*0.06 (0.98)*	**<0.001***
let-7b	*3.22 (19.14)*	*1.98 (4.98)*	**0.012***
let-7c	*3.47 (18.08)*	*2.12 (6.07)*	**0.003***
let-7d	0.41 (1.13)	0.25 (0.70)	0.06
let-7e	*0.83 (3.77)*	*0.47 (1.87)*	**<0.001***
miR-135a	*0.00 (1.41)*	*0.00 (0.15)*	**0.001***
miR-132-3p	*0.04 (0.12)*	*0.02 (0.06)*	**0.001***
miR-139	*0.02 (0.08)*	*0.01 (0.02)*	**<0.001***
Up-regulated			
miR-17	0.03 (0.03)	0.03 (0.15)	0.53
miR-21	1.76 (9.82)	2.88 (10.46)	0.11
miR-34a	0.05 (0.17)	0.06 (0.21)	0.17
miR-141-3p	*0.00 (0.12)*	*0.02 (0.34)*	**0.001***
miR-155	*0.01 (0.24)*	*0.03 (1.39)*	**0.013***
miR-181b	0.02 (0.25)	0.02 (0.50)	0.85
miR-200a	*0.00 (0.11)*	*0.03 (0.29)*	**<0.001***
miR-200b	*0.00 (0.02)*	*0.02 (0.06)*	**<0.001***
miR-200c	*0.09 (1.21)*	*0.52 (4.15)*	**<0.001***
miR-205	*0.00 (8.13)*	*0.03 (4.56)*	**<0.001***
miR-223	*0.00 (0.07)*	*0.01 (0.13)*	**0.001***
miR-375	*0.01 (1.00)*	*0.03 (1.08)*	**0.041***
miR-660	0.01 (0.04)	0.01 (0.09)	0.58
miR-135b	*0.00 (0.01)*	*0.01 (0.08)*	**<0.001***
miR-148b-3p	0.01 (0.33)	0.01 (0.38)	0.95
miR-148a-3p	0.05 (0.09)	0.06 (0.38)	0.11
miR-182	*0.00 (0.00)*	*0.01 (0.17)*	**<0.001***
miR-183	*0.00 (0.00)*	*0.01 (0.08)*	**<0.001***
miR-203-1-3	*0.00 (0.00)*	*0.02 (0.21)*	**<0.001***
miR-429	*0.00 (0.00)*	*0.00 (0.02)*	**0.029***

Differences in miRNA expression levels between endometrioid versus non-endometrioid tumours

We evaluated the difference in miRNA expression level between endometrioid

(EEC) and non-endometrioid (NEEC) tumours. In total, 28 women had endometrioid tumours, 4 serous subtype EC, 2 carcinosarcoma, 1 clear cell and 1 mucinous tumour. There was no difference in expression level of any of the tested miRNAs between EEC and NEEC tumours (**Supplementary Table 2**).

MiRNA to distinguish between low and high-grade endometrioid tumours

To determine if miRNA can accurately distinguish between high-grade (grade 3) and low-grade (grade 1 or 2) EEC, we further analysed women with endometrioid carcinoma histology. In total, 23 women had low-grade EEC and 4 women had high-grade EEC. Only miR-182 had a significant different altered expression pattern (P = 0.041), with median expression level of 0.02 (range 0.15) in high-grade and 0.01 (range 0.03) in low-grade EEC (**Supplementary Table 3**).

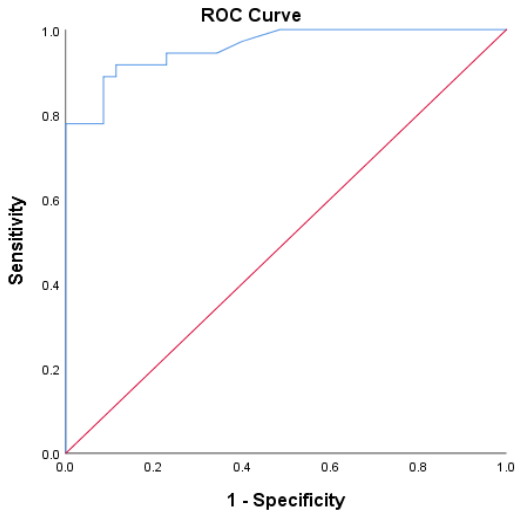


Figure 1. ROC curve for panel of 5 miRNAs (miR-200a, -200b, -200c-, 205 and -182)

Discussion

In this study, we aimed to test and validate the potential of a distinct miRNA panel in FFPE tissue as a first step in optimising it as diagnostic tool for the detection of EC. We demonstrated the highest positive and negative predictive value in diagnosing EC with the distinct panel of miR-200a, miR-200b, miR-200c, miR-205 and miR-182. These results are in line with a study done by Lee et al.[13], which reported a panel of six miRNAs (miR-200a, miR-200c, miR-205, miR-182, miR-183 and miR-21) with an AUC of 0.961, sensitivity and specificity of 91% and 94% respectively in discriminating EC from hyperplasia or benign endometrium.

Different miRNAs have their own distinct mechanism of action with miR-205 being involved in the regulation of PTEN expression, which is the most commonly modified gene in EC[14]. Mutations of PTEN have been described to occur in 25-83% of cases in EC and are frequently present in endometrial hyperplasia[15]. PTEN has an important inhibitory function, promoting apoptosis and proliferation, and its deletion or mutation leads to carcinogenesis[16]. Inactivation of p53 may result in a cellular environment that is contributing to or permissive of oncogenesis[17]. PTEN mutations are more frequently seen in endometrioid type tumours and associated with microsatellite instability, while p53 mutations are more frequently seen in serous or high-grade endometrioid tumours[18,19].

The miR-200 family (which consists of miR-200a, miR-200b, miR-200c, miR-429 and miR-141) has been shown to negatively regulate two transcription factors, ZEB1 (Zinc finger E-box-binding homeobox) and ZEB2, which are well-known transcriptional suppressors of E-cadherin[20]. E-cadherin is a calcium-dependent transmembranous epithelial adhesion molecule involved in the cell cohesiveness. Decreased E-cadherin expression has been linked to decreased cell-cell adhesion, metastatic potential, tumour dedifferentiation and deep myometrial invasion in endometrial and other carcinomas[21]. In EC, loss of E-cadherin is strongly associated with histological subtypes, in which loss is more prevalent in grade 3 EEC compared to serous carcinoma[22]. Furthermore, ZEB1 expression is elevated in NEEC and grade 3 EEC tumours[23].

Finally, miR-182 promotes cell proliferation by targeting the tumour suppressor gene TCEAL7, which interacts with E-box sequences of the Myc target gene cyclin D1, regulating Myc activity and cyclin D1 expression and thus cell proliferation and malignant transformation[24,25].

Although we showed a high diagnostic accuracy for a miRNA panel for EC, we could not discriminate between endometrioid and non-endometrioid tumours. This is in line with results from our systematic review, although numbers were small[9]. However, Ratner et al. showed distinct miRNA signatures for endometrioid tumours, serous carcinomas and carcinosarcomas[26]. Based on the different aetiology of serous and endometrioid EC and subsequent precursor lesions, it is expected that discrimination in the diagnostic approach would be possible. Therefore, further research is needed to understand the biological differences between these types of endometrial cancers and the ability to distinguish between these subtypes of EC.

At the moment, there is interest in preoperative modelling to more accurately detect EC patients with high-grade disease and patients with increased risk of extended disease prior to surgery. A systematic review by Visser et al. showed only

moderate agreement on tumour grade between preoperative endometrial sampling and final diagnosis[6]. Recently, the reproducibility was evaluated with support of The Cancer Genome Atlas (TCGA) classification, yet it was demonstrated that this was mainly supportive in the abnormal p53 group, underlining the relevance of innovative techniques for further improvement. In this study, we found different expression levels of miR-182 for high-grade and low-grade endometrioid tumours, with higher expression levels in high-grade tumours, however numbers were small. A previous study done by Wilcyznski et al. showed lower miR-205 expression rates in grade 3 EEC tumours compared to grade 2 EEC tumours, however Tsukamoto et al. found that miR-205 was up-regulated in poorly differentiated tumours, although again, numbers were limited[27,28]. In addition, Snowdon et al. showed no significant difference in miR-200 family levels between low-grade and high-grade tumours, but also commented their study did not have a significantly large sample size to see such a difference[29]. Further research with larger sample size on pre-operative samples may help establish if miRNA expression as well as a different miRNA panel can accurately distinguish low-grade from high-grade EEC and help improve preoperative diagnosis in order to identify those patients that need extended surgery and/or additional treatment.

The majority of previous studies have used tissue samples to detect miRNA expression levels. However, miRNAs can also be detected in body fluids such as serum and urine[30]. A study by Srivastava et al. showed that a unique miRNA signature can be isolated from the urine of women diagnosed with EC including miR-200c[31]. In addition, Erbes et al. showed the feasibility of urinary miRNA detection in breast cancer patients and its potential as an non-invasive biomarker[32]. Furthermore, studies have shown a similar potential in the usage of serum and plasma[33,34]. These body fluids are promising as a less invasive way of detecting EC and warrant further investigation. Therefore, the panel of miRNA found in this study warrants further evaluation in urine and serum samples to validate its significance in detecting EC.

Altered miRNA expression patterns are not only associated with the development of cancer, but are also linked to various other diseases including obesity, type 2 diabetes mellitus and cardiovascular diseases[35]. This is highly relevant as these are frequently present in patients with EC and hence could reflect underlying comorbidity rather than EC. Several miRNAs have been found to be deregulated in serum of obese women or women with type 2 diabetes, including miR-152, -17, -138, -593, -150, 205, -376a, -432-5p, -500a, and miR-15b[35]. In addition, miRNA is also associated with age, in which certain miRNAs such as miR-151a-3p, miR-181a-5p and miR-1248 were down-regulated in elderly compared to young individuals[36]. MiR-181a expression is found to negatively correlate with the pro-inflammatory cytokines IL-6 and TNFα and positively correlate with the

anti-inflammatory cytokine TGFß. Therefore, this altered expression might be a reflection of the increased inflammation that occurs with advanced age[36]. These miRNAs were not tested in our cohort, therefore further studies are needed to investigate miRNAs that are consistently altered in EC, age and obesity.

Strengths of this study include the inclusion of all histological subtypes and FIGO stages. Furthermore, we have added to the current literature by mainly focusing on miRNAs that have previously been described as promising biomarkers. However, there are limitations to our study, including the retrospective design of the study and the small sample size of EEC patients. In addition, we included samples from hysterectomy specimens and not pipelle samples. Furthermore, there were differences in baseline characteristics between the cancer and control group, which could potentially introduce bias to our results.

Clinical implications and future perspectives

Histopathological examination of endometrial tissue is the gold standard to detect EC. However, miRNA expression in tissue samples can potentially compliment histological diagnosis, aiding in pre-operatively distinguishing between EEC and NEEC and low- and high-grade EEC. In addition, as world population numbers are rising and obesity is becoming more prevalent in undeveloped low resource countries, new technologies that rely less on fully trained pathologists may be of value. Therefore, we believe that investigation and development of alternate diagnostic and/or screening tests may be of value.

Conclusion

Our study showed a high diagnostic accuracy of a distinct miRNA panel of miR-200a, miR-200b, miR-200c, miR-205 and miR-182 to accurately diagnose EC. Furthermore, miR-182 can potentially be used to distinguish high-grade EEC from low-grade disease. MiRNA expression levels in other body fluid such as urine or serum should be further investigated in patients with EC to find a non-invasive screening method for the early detection of EC. Future research is needed to assess and validate miRNA signature in larger, prospective studies.

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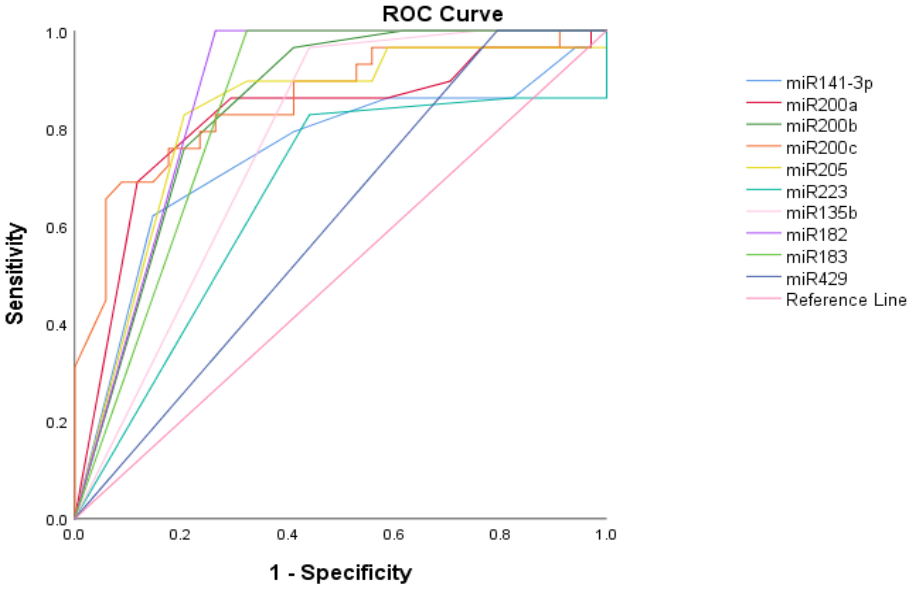
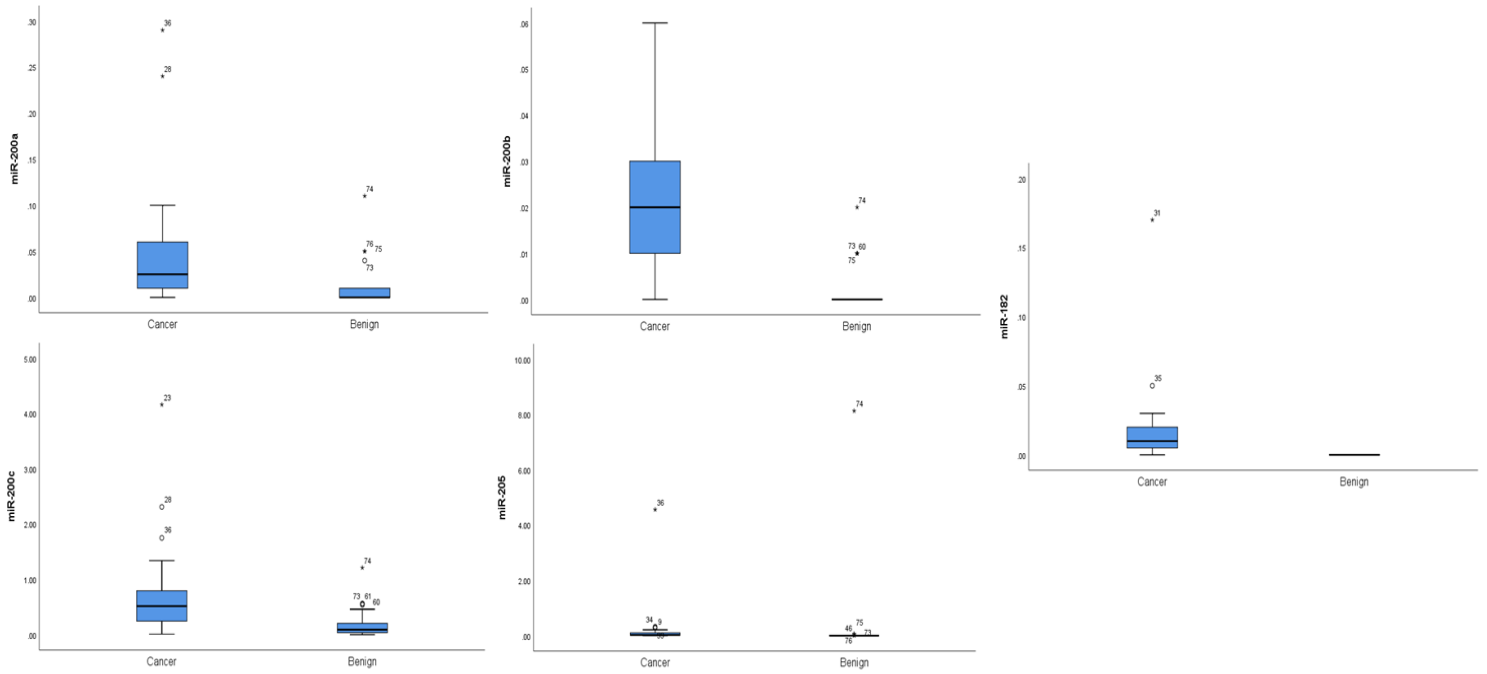
Supplemental material

Supplementary Table 1. Expression values for most promising miRNAs (all up-regulated)

MiRNA	Benign, median (range)	Cancer, median (range)	p-value
miR-141-3p	0.00 (0.12)	0.02 (0.34)	0.001*
miR-200a	0.00 (0.11)	0.03 (0.29)	<0.001*
miR-200b	0.00 (0.02)	0.02 (0.06)	<0.001*
miR-200c	0.09 (1.21)	0.52 (4.15)	<0.001*
miR-205	0.00 (8.13)	0.03 (4.56)	<0.001*
miR-223	0.00 (0.07)	0.01 (0.13)	0.001*
miR-135b	0.00 (0.01)	0.01 (0.08)	<0.001*
miR-182	0.00 (0.00)	0.01 (0.17)	<0.001*
miR-183	0.00 (0.00)	0.01 (0.08)	<0.001*
miR-429	0.00 (0.00)	0.00 (0.02)	0.029*



Supplementary Figure 1. ROC curves for individual miRNAs



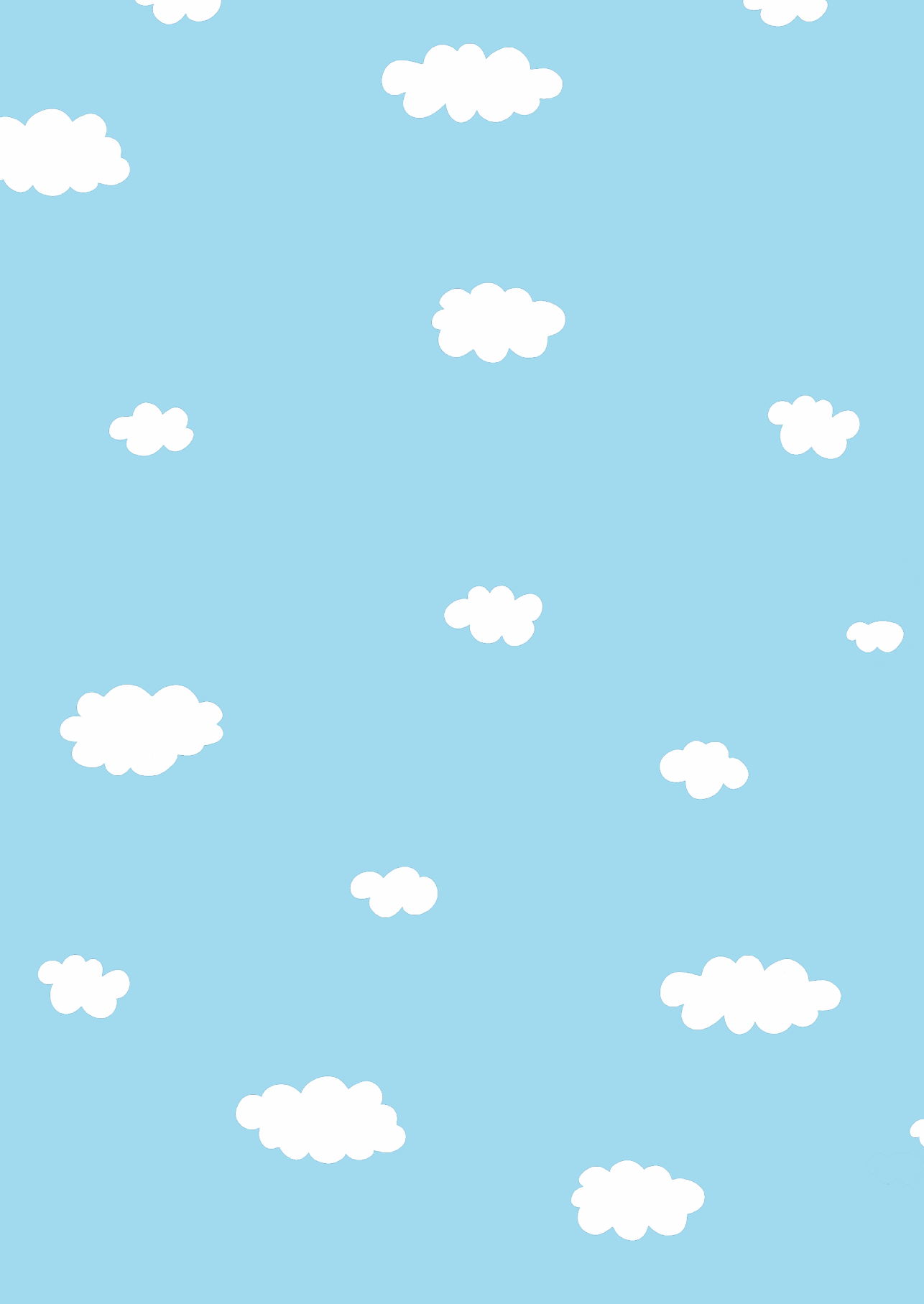
Supplementary Figure 2. Boxplots of expression levels of miR-200a, miR-200b, miR-200c, miR-205 and miR-182

Supplementary Table 2. Expression values for EEC versus NEEC.

MiRNA	EEC Median (range)	NEEC Median (range)	p-value
miR-141-3p	0.03 (0.34)	0.01 (0.07)	0.14
miR-200a	0.04 (0.29)	0.02 (0.07)	0.23
miR-200b	0.02 (0.06)	0.01 (0.03)	0.19
miR-200c	0.54 (4.11)	0.22 (0.71)	0.69
miR-205	0.04 (4.56)	0.02 (0.32)	0.82
miR-223	0.01 (0.04)	0.00 (0.13)	0.79
miR-135b	0.01 (0.08)	0.01 (0.02)	0.64
miR-182	0.02 (0.17)	0.01 (0.05)	0.39
miR-183	0.01 (0.08)	0.01 (0.03)	0.65
miR-429	0.00 (0.02)	0.00 (0.00)	0.32

Supplementary Table 3. Expression values for high-grade versus low-grade EEC

MiRNA	High-grade Median (range)	Low-grade Median (range)	p-value
miR-141-3p	0.02 (0.03)	0.02 (0.21)	1.00
miR-200a	0.06 (0.09)	0.03 (0.29)	1.00
miR-200b	0.04 (0.05)	0.02 (0.04)	0.29
miR-200c	0.54 (0.85)	0.50 (4.11)	1.00
miR-205	0.11 (0.15)	0.03 (4.56)	0.33
miR-223	0.01 (0.02)	0.01 (0.04)	1.00
miR-135b	0.01 (0.08)	0.01 (0.03)	1.00
miR-182	0.02 (0.15)	0.01 (0.03)	**0.041***
miR-183	0.01 (0.07)	0.01 (0.02)	0.50
miR-429	0.01 (0.02)	0.00 (0.02)	0.22



Chapter 9

Detection of microRNA in urine to identify patients with endometrial cancer: a feasibility study

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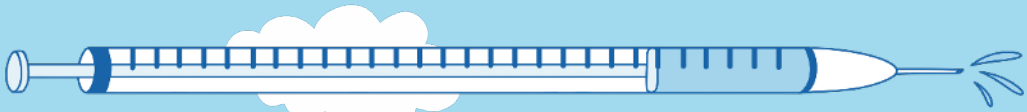
Submitted



Abstract

Introduction

To improve early diagnosis for endometrial cancer (EC), novel non-invasive diagnostic biomarkers are needed. MicroRNAs (miRNAs) are noncoding RNAs that regulate gene expression and contribute to the development of cancer. Therefore, they may be promising novel biomarkers for the detection of EC. Furthermore, miRNAs play an important role in obesity and obesity-related diseases, which are frequently observed among EC patients.

Methods

A prospective cohort study of women presenting with abnormal bleeding between March 2019 and November 2019 was performed at the Royal Cornwall Hospital Trust Truro. Urine samples were obtained from women diagnosed with EC and benign endometrial sampling. MiRNA was isolated and qPCR was used to detect expression levels of miRNAs.

Results

A total of 61 women were included in this study; 24 EC patients and 37 controls. Median age was 64 years and median BMI 29 kg/m^2. MiR-223 was significantly up-regulated in urine of EC patients ($p = 0.003$). Furthermore, let7-i, miR-34a and miR-200c were significantly down-regulated and miR-424 was up-regulated in obese women. In addition, miR-148a and miR-222 were significantly down-regulated in elderly women and miR-16, miR-26b and miR-200c were significantly deregulated in women with multiple comorbidities.

Conclusion

MiRNA expression levels in urine can potentially be used as non-invasive diagnostic test for EC. Furthermore, aberrant miRNA expression in urine is associated with patient characteristics. Further research in larger trials is needed to validate the potential of urinary miRNAs.

Introduction

Endometrial cancer (EC) is the sixth most common gynaecologic cancer in developed countries, accounting for more than 2% of deaths due to cancer worldwide[1]. The prevalence of EC in developed countries has increased in recent years due to the rising prevalence of obesity[1]. Obesity is associated with an increased risk of EC by a variety of mechanisms including increased levels of oestrogen and chronic inflammation leading to hyperinsulinemia and hyperglycaemia, both stimulating endometrial proliferation[2].

The primary symptoms of EC are postmenopausal bleeding (PMB) and abnormal premenopausal bleeding. However, the risk of EC in premenopausal women with abnormal uterine bleeding is low and can be mainly attributed to hormonal imbalances, fibroids, and benign endometrial or cervical polyps[3]. PMB occurs in approximately 90% of women with EC; however, only 9% of women with PMB are diagnosed with EC, with the vast majority being diagnosed with benign conditions such as atrophy, endometrial polyps or unscheduled bleeding in women using hormone replacement therapy (HRT)[4]. As a patient's age increases after menopause, the probability that PMB is caused by EC progressively rises[5–7]. Currently, abnormal uterine bleeding is evaluated with transvaginal ultrasound scan (TVUS) and endometrial biopsy mainly performed by pipelle endometrial sampling. Pipelle endometrial biopsy is an invasive and uncomfortable procedure, with Visual Analogue Scale (VAS) pain score of 6.5 in postmenopausal women[8]. Furthermore, referring all women with abnormal uterine bleeding for TVUS and/or endometrial biopsy carries a considerable cost for healthcare systems. The low positive predictive value of PMB emphasises the need for additional triage tests with high specificity to improve management of abnormal uterine bleeding and avoid unnecessary biopsies in low-risk women. Therefore, novel non-invasive diagnostic biomarkers are needed to improve management, patient care and acceptability of women presenting with abnormal uterine bleeding.

In the past years, microRNAs (miRNAs) have gained enormous interest in cancer research. MiRNAs are a class of small noncoding RNA molecules that regulate several key cellular processes including developmental timing, stem cell division and apoptosis[9]. They play an important role in regulating gene expression; it has been proposed that overexpressed miRNAs may function as oncogenes while those that are underexpressed behave as tumour suppressor genes[10]. Abnormal expression of miRNA has been found in various disorders including numerous cancer sites[11]. MiRNA expression profiles are becoming promising and useful tools in cancer screening, diagnosis and prognosis and may play a future role in cancer therapeutics.

A distinct miRNA panel has shown to be promising in the detection of EC in tissue, plasma or serum samples[12]. In addition, miRNAs have shown to be stable in other body fluids such as pap smear and urine[13]. These body fluids are promising as a non-invasive way of detecting EC.

MiRNAs have emerged as key regulators of lipid and glucose metabolism and play pivotal roles in the onset of obesity and obesity-related diseases[14]. The role of miRNAs as potential link between obesity and EC however, is unknown.

Therefore, the aim of this study is to analyse urinary miRNA expression profiles in EC to find dysregulated miRNAs associated with EC as a first step in finding a non-invasive new diagnostic biomarker. In addition, we aim to determine the correlation of urinary miRNAs with clinicopathological characteristics.

Materials and methods

Study design and participants

A prospective explorative cohort study was performed including women diagnosed with primary EC between March 2019 and November 2019 at the Royal Cornwall Hospital Trust, Truro, United Kingdom.

Recruitment

Women attending their General Practitioner (GP) with abnormal uterine bleeding were referred to PMB clinics for suspected EC. All consecutive women attending PMB clinics were considered eligible and were approached to participate in the study. Detailed information was given and written informed consent was obtained. Those women attending PMB clinics within the described timeframe with histological confirmed benign endometrial tissue served as controls. Urine samples were collected from participants after informed consent following standard protocols and stored at -80°C within 30 minutes after collection until further processing. All included women had the standard investigations of TVUS followed by pipelle endometrial sampling.

Exclusion criteria were: prior or coexisting other malignancies, presence of autoimmune disorders other than diabetes mellitus, inability to provide a urine sample or no endometrial sampling performed.

Standard treatment for women diagnosed with EC was surgically; hysterectomy with bilateral salpingo-oophorectomy, when indicated followed by adjuvant therapy (radiotherapy and/or chemotherapy). The histopathology results were confirmed by surgical resection of tumours in those patients treated with surgery and cancer stage was defined according to the International Federation of

Gynecology and Obstetrics (FIGO) staging criteria[15]. For the 5 (20%) patients who did not undergo surgery, the FIGO stage was purely based on clinical examination and imaging results (CT-scan or MRI-scan and/or PET-CT scan).

The clinical and pathologic variables of the women were extracted from patient records and included age, parity, Body Mass Index (BMI), comorbidities, FIGO stage, histologic subtype and grade.

Sampling and Storage

Urine samples were collected in 100 mL sterile urine sampling cups and frozen in 10 mL aliquots at -80 °C until further processing.

Sample preparation, RNA Isolation, Reverse Transcription and Quantitative real-time PCR (qPCR) of miRNAs

Urine miRNA preparation, RNA isolation and reverse transcription method was described in detail by this group in previous publication[16]. A total of 41 miRNAs were investigated, based on previous literature[12]. **Supplementary Table 1** provides detailed information on the miRNA types studied, including their target sequences.

Statistical analysis

For normal distributed data continuous outcomes were presented as medians and interquartile range, categorical data were presented as frequencies and proportions. For analysis purposes, BMI was categorised into normal (<25 kg/m^2), overweight (25-29.9 kg/m^2), obese (≥30 kg/m^2) and morbidly obese (≥40 kg/m^2). For the data obtained by qRT-PCR, the non-parametric Mann-Whitney U Test was used for the comparison between EC and benign controls and the Benjamini-Hochberg correction was applied to adjust for multiple comparisons with critical value for false discovery rate of 0.25[17]. P-values less than 0.05 were considered significant for all tests. Data were analysed with IBM SPSS statistics version 26.0.

Results

Patient characteristics

All consecutive women that consulted PMB clinics for abnormal bleeding were approached of whom 77 women met the inclusion criteria (**Figure 1**, illustrative flowchart). Out of these women, 74 women consented to participate in the trial. Only three women declined participation due to the following reasons: not interested, no time and fear of identity theft. Subsequently, 13 women were excluded due to histological diagnosis of endometrial hyperplasia (N = 6), cervical cancer (N = 2) or sarcoma (N = 1), due to insufficient RNA extraction (N = 2) or inconclusive histology (N = 2), resulting in a final total of 61 patients suitable for analysis (**Figure 1**).

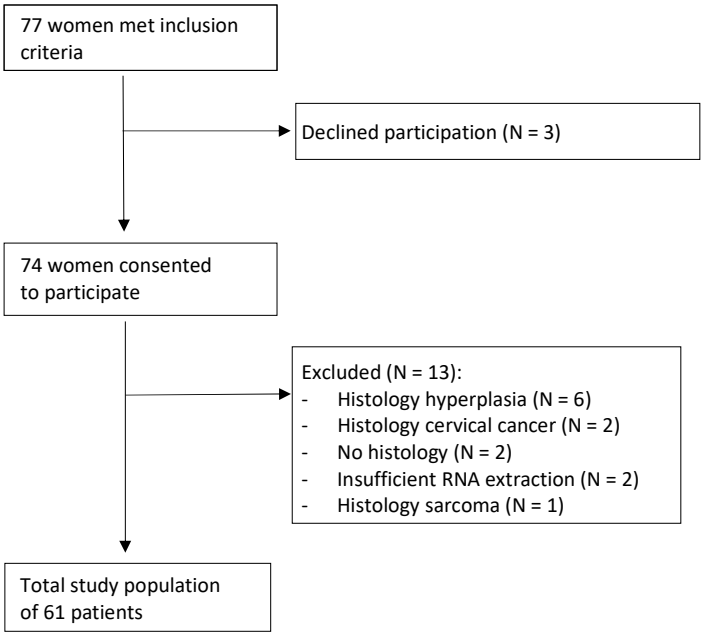


Figure 1. Flowchart of recruitment for study

Median age of patients included was 64 years (Interquartile Range (IQR) 19) and median BMI 29 kg/m^2 (IQR 8) (**Table 1**). There were 24 women diagnosed with EC and 37 women with confirmed benign histology.

The majority of women with EC had endometrioid endometrial carcinoma (EEC; n = 19, 79%), were diagnosed with FIGO stage I (n = 16, 67%) and grade 1 (n = 13, 54%). Most women diagnosed with EC were treated surgically (n = 20, 83%) and four women with advanced-stage disease received hormonal treatment (17%). Histology of women in the control group included atrophic- (n = 22), and proliferative endometrium (n = 15).

Women in the control group were younger with median age of 59 versus 70 years in the EC group, although this did not reach significance (**Table 2**).

Table 1. Baseline characteristics

	Value
No. of patients	61
Age, median (IQR)	64 (19)
BMI, median (IQR)	29 (8)
Ethnicity (%)	
Caucasian	57 (94)
Any other mixed background	2 (3)
Unknown	2 (3)
BMI (%)	
< 25	9 (15)
25 – 30	20 (33)
>30	26 (42)
Unknown	6 (10)
Comorbidities two or more (%)	
Yes	19 (31)
No	38 (62)
Unknown	4 (7)
Histology (%)	
Benign	37 (60)
Endometrioid	19 (31)
Serous	3 (5)
Endometrioid with small area clear cell	1 (2)
Mixed endometrioid / serous	1 (2)
FIGO stage (%)	
I	16 (67)
II	1 (4)
III	3 (13)
IV	1 (4)
Unknown	3 (13)
Grade (%)	
1	13 (54)
2	7 (29)
3	4 (17)
Treatment (%)	
Surgery	20 (83)
Hormonal	4 (17)
Adjuvant therapy (%)	
Yes	6 (25)
No	17 (68)
Unknown	1 (4)

Abbreviations: BMI = Body Mass Index (in kg/m^2), FIGO = International Federation of Gynecology and Obstetrics, IQR = Interquartile Range

MiRNA expression profiles in endometrial cancer

To establish if urinary miRNA can distinguish women with EC from benign endometrium, the differences in expression profiles were assessed. MiR-10b, miR-15a, miR-135b and miR-223 showed significantly altered expression profiles in EC patients (**Table 3**); however only miR-223 remained significantly up-regulated after correction for multiple testing.

Table 2. Differences between cancer and healthy control patients

	Cancer	Benign	p-value
No. of patients	24	37	-
Age, median (IQR)	70 (20)	59 (14)	0.07
BMI, median (IQR)	30 (12)	28 (9)	0.73
BMI (%)			0.12
< 25	1 (4)	8 (22)	
25 – 30	10 (40)	10 (27)	
>30	12 (48)	14 (38)	
Unknown	1 (4)	5 (14)	
Comorbidities two or more (%)			0.57
Yes	9 (38)	10 (27)	
No	15 (62)	23 (62)	
Unknown	0 (0)	4 (11)	
Diabetes Mellitus (%)			0.42
Yes	3 (12)	2 (5)	
No	21 (88)	30 (81)	
Unknown	0 (0)	5 (14)	

Abbreviations: BMI = Body Mass Index (in kg/m^2), FIGO = International Federation of Gynecology and Obstetrics, IQR = Interquartile Range

MiRNA and patient characteristics

To explore the relationship between miRNA and patient characteristics, urinary miRNA expression levels were analysed in relation to BMI, age and comorbidities in the complete study population. All 41 miRNAs were examined out of which eight showed significant different expression patterns (**Table 4**). Let7-i, miR-34a and miR-200c were significantly down-regulated in women with obesity (BMI ≥ 30 kg/m2), whereas miR-424 was up-regulated. In addition, only lower let7-i expression was associated with morbid obesity (BMI ≥ 40 kg/m^2).

The impact of age on miRNA expression was analysed; miR-148a and miR-222 were significantly down-regulated in elderly women. Moreover, miR-16, miR-26b and miR-200c were significantly deregulated in women with multiple comorbidities.

The difference in miRNA expression levels between women with atrophic and proliferative endometrium was assessed, which showed a significant different expression level of let-7a ($p = 0.011$), with a median expression level of 1.04 (range 1.50) in proliferative endometrium and median of 1.50 (range 3.26) for atrophic endometrium (data not shown).

Table 3. Up-regulated and down-regulated miRNAs

	Cancer, median (range)	Benign, range (range)	p-value
Down-regulated			
let-7a	0.99 (2.34)	1.27 (3.26)	0.19
let-7b	2.74 (6.54)	3.06 (16.20)	0.34
let-7c	1.77 (3.75)	2.17 (7.30)	0.21
let-7d	0.15 (0.33)	0.16 (0.55)	0.69
let-7e	0.36 (0.63)	0.40 (1.45)	0.56
let-7i	0.22 (0.50)	0.23 (1.22)	0.62
miR-10b	0.49 (1.40)	0.82 (4.70)	**0.020***
miR-17	0.39 (0.89)	0.48 (1.88)	0.41
miR-20b	0.12 (0.36)	0.19 (1.30)	0.26
miR-26b	1.41 (2.74)	1.83 (3.31)	0.10
miR-100	0.87 (1.87)	1.10 (3.65)	0.10
miR-107	0.38 (0.84)	0.43 (1.07)	0.85
miR-125b	0.31 (1.75)	0.41 (2.81)	0.16
miR-148a	0.41 (1.41)	0.50 (3.00)	0.48
miR-148b	0.07 (0.70)	0.07 (1.00)	0.88
miR-182	0.02 (0.16)	0.03 (2.17)	0.36
miR-183	0.03 (0.13)	0.19 (1.23)	0.78
miR-200a	1.28 (3.11)	1.39 (2.80)	0.73
miR-200b	0.15 (0.46)	0.18 (1.93)	0.59
miR-203-1	1.51 (8.37)	2.06 (16.44)	0.89
miR-222	0.23 (1.75)	0.27 (1.84)	0.41
miR-660	0.18 (0.72)	0.33 (1.15)	0.16
Up-regulated			
miR-15a	0.41 (0.93)	0.30 (0.85)	**0.041***
miR-15b	0.64 (1.42)	0.60 (2.53)	0.59
miR-16	0.71 (1.22)	0.55 (1.68)	0.10
miR-19b	0.90 (1.62)	0.81 (1.88)	0.15
miR-21	9.16 (36.79)	7.21 (49.92)	0.27
miR-34a	0.20 (0.49)	0.12 (0.95)	0.07
miR-135a	0.18 (0.58)	0.13 (0.70)	0.46
miR-135b	0.11 (0.30)	0.06 (0.30)	**0.043***
miR-141-3p	0.48 (1.63)	0.26 (1.31)	0.07
miR-155	0.00 (0.02)	0.00 (0.32)	0.34
miR-181b	0.03 (0.08)	0.02 (0.37)	0.91
miR-195	0.04 (0.10)	0.03 (0.27)	0.50
miR-200c	1.77 (5.55)	1.74 (43.99)	0.77
miR-205	0.99 (6.91)	0.93 (12.61)	0.85
miR-223	0.83 (3.09)	0.20 (3.69)	**0.003***
miR-375	0.12 (0.68)	0.11 (4.00)	0.44
miR-424	0.52 (1.20)	0.46 (20.53)	0.89
miR-429	0.09 (0.21)	0.08 (0.28)	0.58
miR-574	0.21 (1.95)	0.19 (4.70)	0.79

P-values are presented as raw p-values before Benjamini-Hochberg correction

Table 4. MiRNA expression levels and patient characteristics

	let-7i	miR-16	miR-26b	miR-34a	miR-148a	miR-200c	miR-222	miR-223	miR-424
BMI									
< 25 (N = 9)	0.27	0.46	2.19	0.20	0.51	3.42	0.26	0.10	0.38
≥ 25 (N = 45)	0.22	0.57	1.75	0.14	0.44	1.74	0.25	0.47	0.49
p-value	0.67	0.26	0.26	0.16	0.90	0.18	0.45	0.05	0.95
BMI									
< 30 (N = 25)	0.29	0.46	2.19	0.20	0.51	2.37	0.26	0.43	0.32
≥ 30 (N = 29)	0.19	0.64	1.56	0.12	0.42	1.63	0.23	0.44	0.65
p-value	**0.019***	0.41	0.41	**0.041***	0.11	**0.028***	0.09	0.88	**0.022***
BMI									
< 40 (N = 48)	0.25	0.57	1.77	0.16	0.47	1.74	0.26	0.38	0.43
≥ 40 (N = 8)	0.13	0.38	2.68	0.12	0.32	1.89	0.16	0.54	0.66
p-value	**0.010***	0.20	0.20	0.41	0.09	0.58	0.12	0.53	0.56
Age									
< 70 (N = 40)	0.22	0.56	1.78	0.15	0.50	1.74	0.30	0.34	0.46
≥ 70 (N = 21)	0.23	0.64	1.56	0.14	0.40	2.07	0.20	0.47	0.51
p-value	0.70	0.58	0.58	0.98	**0.018***	0.78	**0.025***	0.30	0.57
Two or more comorbidities									
Yes (N = 20)	0.20	0.54	1.86	0.15	0.45	2.37	0.26	0.43	0.40
No (N = 37)	0.27	0.78	1.29	0.18	0.46	1.43	0.28	0.28	0.69
p-value	0.26	**0.023***	**0.023***	0.58	0.62	**0.032***	0.69	0.50	0.22

Abbreviations: BMI = Body Mass Index (in kg/m²)

Discussion

In this study, aberrant expression levels of miR-223 were observed in urine of women with EC. Furthermore, urinary miRNAs were significantly different in obese women (let-7i, miR-34a, miR-200c, miR-424), in elderly (miR-148a, miR-222) and women with multiple comorbidities (miR-16, miR-26b, miR-200c).

MiR-223 has been previously been found deregulated in EC tissue, plasma and serum samples[20] and has a key role in the development and homeostasis of the immune system and its involvement has been demonstrated for many types of cancers, inflammatory diseases and autoimmune diseases[21]. Furthermore, miR-223 is an inflammatory miRNA and is involved in the adipocyte inflammation associated with morbid obesity[22]. In addition, plasma levels of miR-223 were found to be low in patients with type 2 diabetes[23]. Interestingly, we did not observe an association of miR-223 with obesity in our study cohort. This enhances the potential of urinary miR-223 as diagnostic biomarker for EC, even in obese women.

In this study, an association between let-7i, miR-34a, miR-200c, miR-424 and obesity was seen. Previous studies have shown that these miRNAs are associated with insulin production and resistance pathways, pancreatic development and glucose metabolism[24]. To our knowledge, the association between urinary miRNAs and obesity in EC has not been investigated so far. Therefore, the results of our study may help in understanding the role of miRNAs in obesity, metabolism, inflammation and insulin resistance pathways. Urinary miRNAs are promising biomarkers not only in the early detection and screening of EC, but also potentially useful to early identify women at risk of excess body fat accumulation and related metabolic abnormalities.

Currently, literature regarding urinary miRNA expression in EC is scarce. A study by Ritter et al. showed a tendency of miR-10b-5p upregulation and miR-205-5p downregulation in urine of a small set of endometrial-, ovarian- and breast cancer patients[18]. They commented that in these limited number of EC cases, miRNAs could not be confirmed as diagnostic marker in urine samples. In addition, Záveský et al. found urinary miR-106b to be downregulated in 10 EC patients and proposed more research should focus on confirming the diagnostic potential of urinary miRNAs[19].

After the menopause, the endometrium becomes atrophic as a result of ovarian failure. However, a proportion of postmenopausal endometria retain weak proliferative patterns for many years despite being atrophic, mainly as a response to continuous low level oestrogenic stimulation, especially in obese or elderly women[25]. Since normal endometrium is dynamic and undergoing cyclic phenotypic changes, it can be hypothesised that this influences miRNA expression patterns. Based on the miRNAs expression in control women, no differences were observed between proliferative and atrophic endometrium in the miRNA panel we investigated, supporting the fact that this will not influence the discrimination between benign and malignant endometrium. However, age, obesity and comorbidities alter miRNA expression levels and should therefore be take into account in future studies using miRNA expression for improving EC diagnosis.

Lee et al. examined tissue specimens of women with normal endometrial tissue, simple hyperplasia (SH), complex atypical hyperplasia (CAH) and EC and suggested that miR-182, miR-183, miR-200a, miR-200c and miR-205 are involved in progression from CAH to EC, whereas *PTEN* has a role in the progression from SH to CAH[26]. To our knowledge, our study is the first to explore the differences in urinary miRNA expression levels between women with atrophic and proliferative endometrium. In this study, we found a different expression pattern for let-7a between women with atrophic and proliferative endometrium. A further understanding is needed of the role of miRNAs in the development of endometrial

hyperplasia and EC carcinogenesis.

There is limited knowledge on the exact origin of urinary miRNAs. Due to different identification methods and normalisation strategies, the miRNA expression patterns found in studies in other specimens are not easily comparable to urine[29]. Furthermore, the presence of miRNAs in urine differ from those in other bodily fluids such as blood or serum, with lower numbers of detectable miRNA species in the urine[29]. This suggests that the majority of circulating miRNAs are either ''picked up'' by kidneys through an unknown mechanism or are destroyed in urine[30]. Transrenal passage of the miRNAs from the blood could be suspected along with other sources involved in cell-cell communication or passive leakage from the injured or dead cells. Urinary miRNAs might originate from the tumour itself, or could derive from the cells of the immune system as a form of response to the presence of a malignancy[31]. Further research is needed to understand this complex process and comprehend miRNA expression patterns in urine samples of EC patients in more depth.

Strengths and weaknesses

The strengths of this study include the prospective inclusion of PMB patients suspect for EC and the standardised validation of the urinary miRNA expression patterns. However, the study is limited by the small sample size, the inclusion of different subtypes of EC and small groups regarding proliferative or atrophic endometrium. Furthermore, there were differences in baseline characteristics (age) between the cancer and control group. However, we showed that these characteristics were not associated with significant altered expression profiles of miR-223, therefore we assume this has not affected our results. In addition, miRNA expression patterns can be correlated with stage at diagnosis, however due to small numbers we were unable to account for stage.

Implications for practice and future research

This study was performed to establish the feasibility of urinary miRNA testing in EC as a first step in finding a non-invasive new diagnostic biomarker to improve management of women presenting with abnormal uterine bleeding. Ideally, a urinary screening test for those women can easily be done at the General Practitioner and only women with abnormal screening test can then be send to a hospital for further investigations, thereby improving patient care and early diagnosis. Further research in larger prospective trials should focus on validating the potential of urinary miRNA as diagnostic biomarker.

Conclusion

In the current study, we demonstrated the feasibility of using miRNA expression levels in urine as possible non-invasive diagnostic test for EC. The miRNA levels can contribute to improved understanding of the carcinogenesis and its relation to BMI and comorbidity. Further research in larger trials is needed to validate the potential of these miRNAs.

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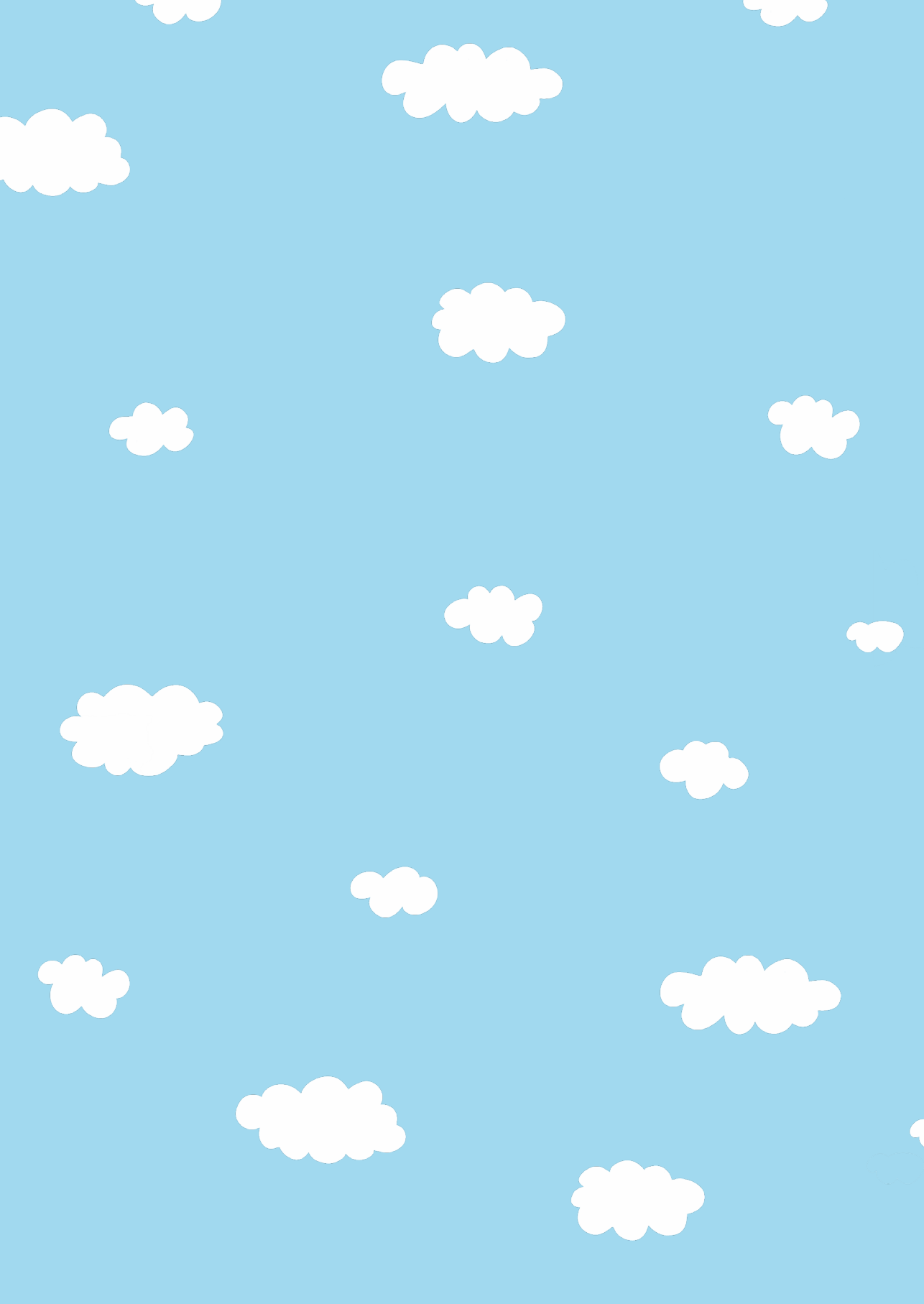
Supplemental material

Supplementary Table 1. Information on miRNA names, sequences and primers utilized in expression analysis.

miRNA	miRNA sequence	sense primer	antisense primer
cel-miR39-3p#	UCACCGGGUGUAAAUCAGCUUG	GTCACCGGGTGTAAATCAG	GGTCCAGTTTTTTTTTTTTTTCAAG
ath-miR159a#	UUUGGAUUGAAGGGAGCUCUA	GCGCAGTTTGGATTGAAG	AGGTCCAGTTTTTTTTTTTTTTAGAG
hsa-let7a-5p	UGAGGUAGUAGGUUGUAUAGUU	GCAGTGAGGTAGTAGGTTG	GGTCCAGTTTTTTTTTTTTTTAACTATAC
hsa-let7b-5p	UGAGGUAGUAGGUUGUGUGGUU	CAGTGAGGTAGTAGGTTGTGT	GGTCCAGTTTTTTTTTTTTTTAACCA
hsa-let7c-5p	UGAGGUAGUAGGUUGUAUGGUU	GCAGTGAGGTAGTAGGTTGT	GGTCCAGTTTTTTTTTTTTTTAACCA
hsa-let7d-5p	AGAGGUAGUAGGUUGCAUAGUU	CGCAGAGAGGTAGTAGGTTG	GGTCCAGTTTTTTTTTTTTTTAACTATG
hsa-let7e-5p	UGAGGUAGGAGGUUGUAUAGUU	GCAGTGAGGTAGGAGGTTG	GGTCCAGTTTTTTTTTTTTTTAACTATAC
hsa-let7i-5p	UGAGGUAGUAGUUUGUGCUGUU	GCAGTGAGGTAGTAGTTTGTG	GGTCCAGTTTTTTTTTTTTTTAACAG
hsa-miR10b-5p	UACCCUGUAGAACCGAAUUUGUG	CAGTACCCTGTAGAACCGAAT	GGTCCAGTTTTTTTTTTTTTTCAC
hsa-miR15a-5p	UAGCAGCACAUAAUGGUUUGUG	CAGTAGCAGCACATAATGGT	GGTCCAGTTTTTTTTTTTTTTCACA
hsa-miR15b-5p	UAGCAGCACAUCAUGGUUUACA	GCAGTAGCAGCACATCA	CCAGTTTTTTTTTTTTTTTGTAAACCA
hsa-miR16-5p	UAGCAGCACGUAAAUAUUGGCG	CGCAGTAGCAGCACGTA	CAGTTTTTTTTTTTTTTCGCCAA
hsa-miR17-5p	CAAAGUGCUUACAGUGCAGGUAG	GCAAAGTGCTTACAGTGCAG	GGTCCAGTTTTTTTTTTTTTTCTAC
hsa-miR19b-3p	UGUGCAAAUCCAUGCAAAACUGA	AGTGTGCAAATCCATGCAA	GGTCCAGTTTTTTTTTTTTTTCAGT
hsa-miR20b-5p	CAAAGUGCUCAUAGUGCAGGUAG	GCAAAGTGCTCATAGTGCAG	GGTCCAGTTTTTTTTTTTTTTCTAC
hsa-miR21b-5p	UAGCUUAUCAGACUGAUGUUGA	GCAGTAGCTTATCAGACTGATG	GGTCCAGTTTTTTTTTTTTTTCAAC
hsa-miR26b-5p	UUCAAGUAAUUCAGGAUAGGU	CGCAGTTCAAGTAATTCAGGAT	GGTCCAGTTTTTTTTTTTTTTACCT
hsa-miR34a-5p	UGGCAGUGUCUUAGCUGGUUGU	GCAGTGGCAGTGTCTTAG	GGTCCAGTTTTTTTTTTTTTTACAAC
hsa-miR100-5p	AACCCGUAGAUCCGAACUUGUG	CAGAACCCGTAGATCCGA	GTCCAGTTTTTTTTTTTTTTCACAAG
hsa-miR107-5p	AGCAGCAUUGUACAGGGCUAUCA	GCAGAGCAGCATTGTACAG	GGTCCAGTTTTTTTTTTTTTTGATAG
hsa-miR125b-5p	UCCCUGAGACCCUAACUUGUGA	GCAGTCCCTGAGACCCT	CCAGTTTTTTTTTTTTTTCACAAGT
hsa-miR135a-5p	UAUGGCUUUUUAUUCCUAUGUGA	CGCAGTATGGCTTTTTATTCCT	GGTCCAGTTTTTTTTTTTTTTCACA
hsa-miR135b-5p	UAUGGCUUUUCAUUCCUAUGUGA	GCAGTATGGCTTTTCATTCCT	GGTCCAGTTTTTTTTTTTTTTCACA
hsa-miR141-3p	UAACACUGUCUGGUAAAGAUGG	CGCAGTAACACTGTCTGGT	GTCCAGTTTTTTTTTTTTTTCCATCT
hsa-miR148a-3p	UCAGUGCACUACAGAACUUUGU	GCAGTCAGTGCACTACAGA	GGTCCAGTTTTTTTTTTTTTTACAAAG
hsa-miR148b-3p	UCAGUGCAUCACAGAACUUUGU	GCAGTCAGTGCATCACAGA	GGTCCAGTTTTTTTTTTTTTTACAAAG
hsa-miR155-5p	UUAAUGCUAAUCGUGAUAGGGGU	CGCAGTTAATGCTAATCGTGATAG	AGGTCCAGTTTTTTTTTTTTTTACC
hsa-miR181b-5p	AACAUUCAUUGCUGUCGGUGGGU	GCAGAACATTCATTGCTGTC	GTCCAGTTTTTTTTTTTTTTACCCA

table continues

miRNA	miRNA sequence	sense primer	antisense primer
hsa-miR-182-5p	UUUGGCAAUGGUAGAACUCACACU	GTTTGGCAATGGTAGAACTCA	GGTCCAGTTTTTTTTTTTTTTTAGTGT
hsa-miR-183-5p	UAUGGCACUGGUAGAAUUCACU	GCAGTATGGCACTGGTAGA	GGTCCAGTTTTTTTTTTTTTTTAGTGA
hsa-miR195-5p	UAGCAGCACAGAAAUAUUGGC	CGCAGTAGCAGCACAGA	TCCAGTTTTTTTTTTTTTTTGCCA
hsa-miR200a-3p	UAACACUGUCUGGUAACGAUGU	CAGTAACACTGTCTGGTAACG	GGTCCAGTTTTTTTTTTTTTTTACATC
hsa-miR200b-3p	UAAUACUGCCUGGUAAUGAUGA	CGCAGTAATACTGCCTGGT	GGTCCAGTTTTTTTTTTTTTTTCATCA
hsa-miR200c-3p	UAAUACUGCCGGGUAAUGAUGGA	AGTAATACTGCCGGGTAATGA	GGTCCAGTTTTTTTTTTTTTTTCCA
hsa-miR-203-1 3p	GUGAAAUGUUUAGGACCACUAG	CAGGTGAAATGTTTAGGACCA	GGTCCAGTTTTTTTTTTTTTTTCTAGT
hsa-miR205-5p	UCCUUCAUUCCACCGGAGUCUG	CCTTCATTCCACCGGAGT	GGTCCAGTTTTTTTTTTTTTTTCAGA
hsa-miR375-5p	UUUGUUCGUUCGGCUCGCGUGA	AGTTTGTTCGTTCGGCTC	GGTCCAGTTTTTTTTTTTTTTTCAC
hsa-miR424-5p	CAGCAGCAAUUCAUGUUUUGAA	AGCAGCAGCAATTCATGT	AGGTCCAGTTTTTTTTTTTTTTTCAA
hsa-miR429-5p	UAAUACUGUCUGGUAAAACCGU	CGCAGTAATACTGTCTGGT	GTCCAGTTTTTTTTTTTTTTTACGGT
hsa-miR574-3p	CACGCUCAUGCACACACCCACA	ACGCTCATGCACACAC	GTCCAGTTTTTTTTTTTTTTTGTGG
hsa-miR660-5p	UACCCAUUGCAUAUCGGAGUUG	AGTACCCATTGCATATCGGA	GGTCCAGTTTTTTTTTTTTTTTCAAC
miRNA RT*		CAGGTCCAGTTTTTTTTTTTTTTTVN	



Chapter 10

General discussion and future perspectives

General discussion and conclusion

In this thesis, different factors influencing endometrial cancer (EC), outcomes and its relationship with obesity were described. In addition, the diagnostic value of miRNA detection in EC in tissue and urine samples was evaluated. The relevance and clinical implications of the thesis will be discussed in this chapter, along with recommendation for future research.

Obesity is a complex and multifactorial problem, mainly in the Western world, and a significant risk factor for and contributor to increased morbidity and mortality[1]. It has been well-established that EC risk is strongly related to obesity with a clear relationship between obesity and EC incidence and carcinogenesis. Although obesity as risk factor has been mainly attributed to endometrioid type EC (EEC), recent data illustrate that obesity is also relevant in non-endometrioid type EC (NEEC)[2]. However, questions regarding obesity-related endometrial carcinogenesis remain.

Obesity and body composition

Body Mass index (BMI) is a convenient and accessible measure of weight in the clinical setting. However, it does not differentiate between visceral or subcutaneous fat and muscle mass[3,4]. It is becoming increasingly apparent that the distribution of fat tissue is at least as important as the total amount of body fat in predicting disease-causing complications that are associated with obesity[5]. Visceral obesity is linked to metabolic alterations such as insulin resistance, type 2 diabetes, hypercholesterolaemia, cardiovascular disease and chronic systemic inflammation leading to cancer development and progression[6]. Furthermore, in addition to the importance of fat distribution, reduced muscle mass is a known risk factor for poor clinical outcomes such as post-operative complications, chemotherapy-related toxicity and overall survival (OS) in patients with cancer[7]. Sarcopenia refers to the loss of muscle mass and strength and is associated with older age[8]. Sarcopenia is clinically important as it is a major risk factor for physical frailty, falls, prolonged hospitalisation, dependency and premature mortality[9]. Sarcopenia is often seen in obese individuals, a condition called sarcopenia obesity, with a prevalence of up to 20% in older populations[10].

Body composition in EC

We explored the relationship between body composition including fat and muscle distribution and outcomes in high-grade EC. In **Chapter 2**, we showed that high visceral fat percentage (VAV%) is associated with reduced OS and disease-specific survival (DSS) in NEEC, but not in EEC. This is an unexpected finding and raises questions about the pathophysiology of EC carcinogenesis. Setiawan et al. found risk factors for NEEC tumours to be similar to EEC, suggesting NEEC tumours are

not completely oestrogen independent, as previously thought[2]. Van Weelden et al. furthermore showed a stronger correlation of subcutaneous fat volume (SAV) with oestradiol when compared to visceral fat volume (VAV), hypothesising both SAV and VAV are relevant for EC carcinogenesis[11]. SAV is primarily responsible for oestradiol production and therefore related to endometrial hyperplasia and EEC tumours, whereas VAV is involved in alterations leading to hyperinsulinemia and inflammation-related carcinogenesis[11]. We therefore expected VAV% to be associated with survival in both NEEC and EEC tumours. Mauland et al. add to our findings showing high VAV% is associated with worse DSS, however they included mainly patients with low-grade EC (152 grade 1-2 EEC)[12]. It can therefore be hypothesised VAV% is only prognostically relevant in low-grade EEC tumours, but not in high-grade EEC as shown by our study. Our findings underline that, although it is becoming increasingly more apparent NEEC are also related to obesity, there still seems to be a difference in pathophysiology between the obesity-related NEEC and EEC.

Furthermore, we showed the association of VAV% with hypertension and diabetes mellitus (DM) in high-grade EC. This association was seen for both NEEC and EEC tumours. The mechanism behind an increased risk of hypertension associated with obesity lies in an altered angiotensin II and aldosterone secretion system associated with excess weight, mainly visceral fat[13,14]. Furthermore, obesity causes structural alterations in the kidneys that eventually cause loss of nephron function and a further elevation in blood pressure[13]. In addition, accumulation of visceral fat contributes to adipocyte hypertrophy and increased infiltration with macrophages, which both secrete several adipokines, creating a complex network of factors promoting, maintaining and exacerbating an insulin resistant state[15].

The importance of body fat distribution maybe evident; however, questions remain regarding the exact impact of body composition in EC progress. Therefore, examining muscle mass in addition to fat distribution is important. Muscle mass has been shown to have a protective effect on cardiometabolic alterations in obesity[16]. On the other hand, obesity leads to muscle atrophy and decreased muscle strength, worsening the adverse effects of obesity[17,18]. We showed that sarcopenia is not associated with reduced survival in patients with high-grade EC in **Chapter 3**. This is supported by previous data of Nattenmüller et al. and Kuroki et al. showing no impact of sarcopenia on OS[19,20]. However, Kuroki et al. showed a negative impact of sarcopenia on recurrence-free survival[20]. Furthermore, we revealed an association between sarcopenic obesity and poor OS in high-grade EEC. This association was not seen for NEEC patients and might be contributed to the limited number of cases.

Risk factors for the pathogenesis of sarcopenia include older age, female sex, low levels of physical activity or immobility[21]. At the cellular level, the age-related loss of

muscle mass that occurs in sarcopenia is caused by a decrease in both the number and size of muscle fibres[9]. This is an age-related process due to oxidative damage, low-grade chronic inflammation, nutritional factors and changes in hormonal system[9]. Studies on body composition in other tumour types showed an impact of sarcopenia on outcomes including survival[22]. This illustrates that underlying pathogenic mechanisms are complex and not fully understood. Prospective studies are needed to evaluate the composition of adipose tissue as well as muscle mass and its influence on prognosis in EC while addressing changes over time. In addition, menopause status should be taken into account, since menopause and bilateral oophorectomy are associated with an increase in percentage body fat[23].
To improve outcome in women diagnosed with EC, issues of obesity and sarcopenia should be addressed. Several treatment regimes have been proposed to improve visceral adiposity and increase muscle mass, including exercise, diet and medication. Resistance exercise remains the intervention with the most supporting evidence for the effectiveness to increase muscle mass and is the treatment of choice[24]. There is currently no recommended pharmacological treatment, however trials have examined the usage of inhibitors of the myostatin system, testosterone and angiotensin-converting enzyme inhibitors[25,26].

The importance of visceral adiposity as well as muscle loss seems evident and we propose including body composition measurements as part of standard work-up for patients newly diagnosed with EC. In addition, we suggest EC patients should be offered specialised training programs and nutritional therapy to aim to achieve weight loss including reducing visceral fat volumes and to increase muscle mass.

Socioeconomic status, obesity and EC

Obesity is a multifactorial problem in which individual, environmental, socioeconomic and genetic factors are all linked to the increase in obesity in developed countries[27]. Obesity is more frequently seen amongst individuals with lower socioeconomic status (SES) due to less physical activities, unhealthy working- and living environment, unhealthier diet due to costs and lack of knowledge regarding healthy food and psychological distress[28–30]. Socioeconomic deprivation is an important predictor of health and survival with major inequalities among different levels of deprivation[31,32]. Extensive literature describes the relationship between socioeconomic deprivation and poor cancer survival[33]. In **Chapter 4 and 5**, we have shown that socioeconomic deprivation is associated with worse survival in EC patients (as shown by our systematic review of the literature, **Chapter 4**), however this is attributed to other factors such as obesity, higher stage at diagnosis and age (as shown by our own retrospective cohort study, **Chapter 5**). However, the independent relationship between obesity and OS was only seen in EEC patients, but not in NEEC patients. In NEEC patients, stage at diagnosis was the most important prognostic factor.

The majority of EC patients present early with an overall good prognosis. However, low SES patients generally undergo a longer time interval between first symptom recognition and first medical consultation due to low awareness of symptoms and less ability to afford medical care[34]. Furthermore, low educational status is associated with referral delay[35]. This health-care delay is associated with more advanced stage disease at presentation[34]. Furthermore, socioeconomic differences in treatment modalities have been reported, with more aggressive therapy in patients with high SES[34]. Reasons for this association include differences in attitudes towards invasive procedures, disease severity, access to care, differences in undergoing staging procedures and issues related to health insurance[34].

Therefore, early diagnosis is the most important adjustable factor in EC to improve outcomes. Increasing public awareness on the association between obesity and cancer including breast-, colon- and endometrial cancer and early symptoms of disease is important. The economic burden of obesity-related cancers is substantial and is expected to grow since the prevalence of obesity increases[36]. For that reason, interventions for implementing obesity prevention or treatment programs among the general population and cancer patients could have an impact on outcomes as well as overall cancer care expenditures. Public health interventions include education and prevention including hormonal protection in obese women or bariatric surgery. Among obese women diagnosed with complex atypical hyperplasia (CAH) or early-stage EC, referral to a bariatric surgeon tends to be well received, with approximately 17% going on to pursue surgical intervention and 59% subsequently attempting weight loss[37]. Furthermore, MacKintosh et al. showed that in obese women, bariatric surgery-induced weight loss resulted in beneficial changes in circulating biomarkers of insulin resistance, inflammation, and reproductive hormones, in endometrial morphology, and in molecular pathways that are implicated in endometrial carcinogenesis[38].

Obesity and haematological parameters in EC

Obesity is associated with chronic low-grade inflammation with elevated levels of pro-inflammatory cytokines and acute phase proteins[39]. Excessive pro-inflammatory cytokine production stimulates the release of platelets from bone marrow to peripheral blood and these cause changes in haematological parameters[40]. In **Chapter 6**, we showed the prognostic relevance of haematological parameters and its relationship with obesity and comorbidities in women diagnosed with EC. We showed an independent relationship of anaemia with poor OS. Furthermore, we showed that leucocytosis was associated with obesity, DM and advanced FIGO stage. These results indicate leucocytosis is related to unhealthy patient environment and therefore related to adverse outcome, whereas anaemia reflects tumour aggressiveness. These haematological parameters can be useful in further prognostication of EC patients.

MicroRNA, cancer and obesity

As summarised, alterations in body composition are highly relevant in both the incidence and the outcome of EC. Although prevention has high priority, improving diagnosis might be as important. Early identification of precancerous and early-stage disease is therefore important, and less invasive diagnostics could facilitate early diagnosis. Therefore, the second part of the thesis focused on identifying a novel non-invasive test for the detection of EC.

In the past years, microRNAs (miRNAs) have gained enormous interest in cancer research. MiRNAs are a class of small noncoding RNA molecules that regulate gene expression which play crucial roles in biological processes[41,42]. Abnormal expression of miRNA has been linked to various disorders including cancer and have therefore been proposed to be promising diagnostic and prognostic biomarkers[43,44]. Additionally, miRNAs are associated with lipid and glucose metabolism and play an important role in the onset of obesity and obesity-related diseases[45].

MiRNAs have shown to be stable in body fluids such as serum and urine[46]. These body fluids are promising as a non-invasive way of detecting EC. Ideally, a screening test for EC could be done at the general practitioner (GP). Only women with abnormal screening test can then be send to a hospital for further investigations. This would lower thresholds for patients and could therefore improve early diagnosis. In addition, access to and use of health care vary by SES. Even in countries that provide universal coverage, persons with less income and education do not use health services in the same way as wealthier individuals do[47]. People with lower SES use GP services more frequently while individuals with higher SES more often use specialist medical services[48]. A urinary screening test for EC could improve early diagnosis in patients with low SES.

MiRNA as diagnostic biomarker in EC

We showed the potential of miRNA as a diagnostic biomarker in EC in **Chapter 7**. We performed a systematic review of the literature and found that miR-200a, miR-200b, miR-200c, miR-205, miR-141, miR-429, miR-182, miR-183 and miR-223 were most consistently upregulated in various specimens (tissue, urine, liquid-based cytology, serum, plasma) in women diagnosed with EC.

We therefore further analysed this specific miRNA panel in our own cohort in tissue samples of women diagnosed with EC to further validate the potential of miRNA as a diagnostic biomarker. We showed a high diagnostic accuracy in detecting EC with a distinct panel of miR-200a, miR-200b, miR-200c, miR-205 and miR-182 with sensitivity of 92% and specificity of 89% in distinguishing benign endometrial tissue from EC (**Chapter 8**).

As previously stated, miRNAs are stable in urine, opening a potential way for non-invasive testing for EC. Due to different identification methods and normalisation strategies, miRNA expression patterns found in other specimens are not easily comparable to urine[49]. Furthermore, the presence of miRNAs in urine differ from those in other bodily fluids such as blood or serum, with lower numbers of detectable miRNA species in the urine[49]. In **Chapter 9**, we investigated miRNA expression levels in urine samples of EC patients and benign control women and showed that miR-223 levels were up-regulated in urine samples of women diagnosed with EC. We therefore showed the feasibility of urinary miRNA testing as a first step in finding a non-invasive biomarker for the screening and detection of EC. Further, larger prospective trials are needed to validate the potential of these miRNAs in the accurate diagnosis of EC. Not only urine is a potential non-invasive way to detect or screen for EC, other samples such as serum or pap smear could potentially be used and the presence of miRNAs in these samples warrant further investigations.

We found certain urinary miRNAs to be associated with BMI: let-7i, miR-34a, miR-200c and miR-424. Additionally, Munetsuna et al. revealed the association between circulating miRNAs and SAT and VAT[50]. Therefore, miRNAs may be useful as biomarker for obesity and offer new information to better understand the pathogenesis of obesity. They could further aid in explaining gaps in the existing knowledge of the obesity-related endometrial carcinogenesis and differences between NEEC and EEC tumours.

Future directions

This thesis described and highlights the importance of understanding obesity-related endometrial carcinogenesis. Although we have shown that EEC differ from NEEC with regards to prognostic factors, both seem to be related to obesity. Future research should focus on this incomplete understanding while incorporating The Cancer Genome Atlas (TCGA) molecular classification. There appears to be an association between obesity and the EC integrated clusters, in which *POLE* mutated tumours have the lowest BMI and women with copy-number low tumours have the highest median BMI[51]. However, the association with body composition is unknown. Since it's becoming more apparent that body composition is equally or perhaps even more important than BMI, future studies should focus on, and take into account body measurements.

We have shown that visceral fat negatively impacts survival in NEEC, however it would be clinically useful to establish a prognostic cut-off value of VAV%. Studies have been conducted to suggest thresholds of VAT associated with

low cardiometabolic risk, but criteria were highly variable across studies[6]. To propose such cut-off risk values, it will be critical to standardise the location of the abdominal CT-scan or to clearly identify the abdominal cross-sectional area (e.g. L4-L5 or umbilicus) that was used. A prognostic cut-off value for EC could help clinicians assess patients at increased risk for poor outcome and establish which patients should be counselled for specialised rehabilitation and training programs including nutritional therapy. The effects of such lifestyle interventions on reducing visceral fat and gaining muscle mass in women diagnosed with EC have not yet been assessed and could aid in the development of a comprehensive combined lifestyle intervention program while taking into account the frequent comorbidities, disabilities and older age EC patients have. This will also help answer one of the top 10 unanswered research questions developed by Wan et al. in 2016: ''Do changes in lifestyle, including weight loss, reduce the risk of recurrence and improve survival in women who have been treated for endometrial cancer?''[52]. We furthermore revealed that high-grade EEC patients with sarcopenic obesity have poorer survival, further emphasising the need for posttreatment rehabilitation. Lee et al. previously showed that loss of muscle during cancer therapy was predictive for poor survival in advanced EC, however it would be of interest to investigate if similar results can be found in early-stage EC with usually better prognosis[53]. Furthermore, the optimal intervention that best preserves skeletal muscles has yet to be determined. A program with combined resistance and aerobic exercises significantly attenuated sarcopenic obesity in patients with breast cancer, however for women with EC, who are often elderly, this has not been investigated[54].

Not only postsurgical therapy is important, establishing and optimising patients' preoperative health status is important. The MUST (Malnutrition Universal Screening Tool) is used in several hospitals in the Netherlands to preoperatively determine nutritional risk and to identify adults who are malnourished, at risk of malnutrition, or obese[55]. It also includes management guidelines which can be used to develop a care plan. The value of this screening tool, however, has not been explored for women diagnosed with EC. Furthermore, the time period between diagnosis and surgical intervention can be used to optimise patients' status in a prehabilitation program. Multimodal prehabilitation programs could include nutritional counselling, training programs, psychological support and optimisation of underlying medical conditions as well as cessation of unfavourable health behaviours such as smoking and drinking and could optimise patients overall health before EC surgery.

Body composition including fat distribution and muscle mass are important in incidence and outcome of EC. With regards to further improving early diagnosis in EC and thereby improving survival rates, this thesis aimed to find and describe a new non-invasive biomarker. Although we showed the feasibility of using urinary

miRNA expression levels in diagnosing EC, we recognise the limitations of our study with respect to the small sample size. Therefore, we aim to validate our findings in a larger prospective trial. We furthermore would like to include women diagnosed with simple hyperplasia and complex atypical hyperplasia, to verify if miRNAs can distinguish benign endometrium from hyperplasia and hyperplasia from cancer. This could then potentially be useful in management of mainly obese women with findings of hyperplasia with or without atypia.

Additionally, we showed the association between obesity and biomarkers (urinary miRNAs and haematological parameters), however we have not assessed the value of miRNA or haematological parameters as marker for body composition. If certain markers are associated with the presence of sarcopenia or visceral adiposity, they could be used to early identify women at risk of body fat accumulation or loss of muscle mass and related metabolic abnormalities. Compared to CT-scan, the gold standard for assessing body composition, urinary miRNAs and haematological parameters are non-invasive and less expensive. These biomarkers could easily be used to assess the influence of lifestyle including rehabilitation therapy.

Conclusion

Obesity influences survival and outcomes in EC in different ways. Not only BMI is important in EC prognosis, but body fat composition including visceral fat and muscle mass are vital. High visceral fat is an independent prognostic factor for women with NEEC tumours, whereas sarcopenic obesity is associated with poor survival in high-grade EEC patients. In addition, socioeconomic deprivation is associated with poor survival, however this is attributed to other factors such as obesity. Furthermore, anaemia is an independent prognostic factor in EC, whereas leucocytosis is related to obesity and comorbidity.

MiRNAs are related to obesity and are promising biomarkers in detecting EC. Moreover, urinary miRNAs could potentially be useful as a non-invasive diagnostic biomarker, however this should be further investigated in larger trials.

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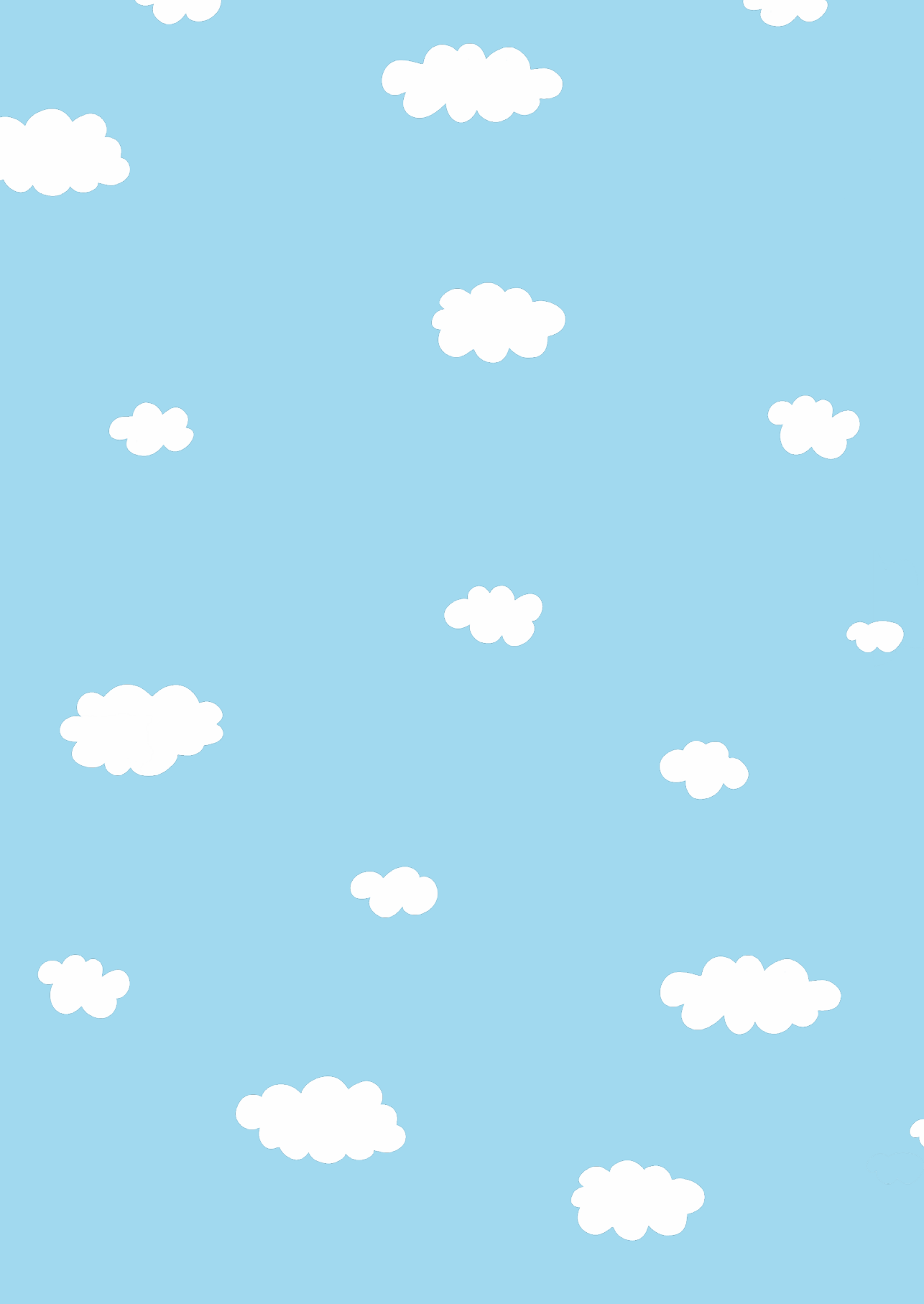


Chapter 11

Summary
Samenvatting

Summary

Endometrial cancer (EC) is the most common gynaecological malignancy in the Western world. As described in **Chapter 1**, these carcinomas are traditionally classified as either endometrioid adenocarcinomas (EEC, associated with obesity) or non-endometrioid tumours (NEEC, seen in the presence of endometrial atrophy and presumed non-related to obesity). However, recent data illustrate that risk factors associated with obesity are also relevant in NEEC tumours. Nevertheless, questions remain regarding the obesity-related endometrial carcinogenesis.

To further explore the influence of obesity on EC and outcomes, **Chapter 2** and **3** of this thesis focussed on the relationship between body fat measurements and outcomes in EC. In **Chapter 2**, body fat distribution was measured with CT-scan and analysed in relationship to mortality in high-grade EC patients. We showed that high visceral fat percentage (VAV%) was associated with reduced overall- (OS) and disease-specific survival (DSS) in NEEC, but not in high-grade EEC. Furthermore, we showed the association of VAV% with hypertension and diabetes mellitus (DM) in high-grade EC.

In addition to body fat distribution, examining muscle mass is important. In **Chapter 3**, we analysed sarcopenia, defined as loss of skeletal muscle, and found sarcopenia to be unrelated to survival in patients with high-grade EC. However, we did show an association between sarcopenic obesity and poor OS in high-grade EEC, although this association was not seen for NEEC patients. We therefore propose to incorporate body composition measurements, in addition to Body Mass Index (BMI), as part of standard work-up to be able to offer patients specialised training programs and nutritional therapy aiming at reducing visceral fat volumes and increasing muscle mass, thereby improving outcomes in EC.

Obesity is a multifactorial problem and is more frequently seen amongst individuals with lower socioeconomic status (SES). Hence, we focused on the relationship between socioeconomic deprivation, BMI and survival in EC. Socioeconomic deprivation was associated with worse survival in EC, as shown by our systematic review of the literature (**Chapter 4**). However, we conducted a retrospective cohort study in our own institution and showed that these survival differences were attributed to other factors such as obesity, higher stage at diagnosis and age (**Chapter 5**). The independent relationship between obesity and OS was only seen in EEC patients. In NEEC patients, stage at diagnosis was the most important prognostic factor.

Since obesity is associated with chronic low-grade inflammation with elevated levels of pro-inflammatory cytokines and acute phase proteins, we analysed

haematological parameters in relationship to survival in EC[38]. In **Chapter 6**, we showed the importance of haematological parameters and its relationship with obesity and comorbidities. Anaemia is an independent prognostic factor whereas leucocytosis is related to obesity and comorbidity; reflecting an unhealthy patient environment. We concluded that these markers can be useful in further prognostication of EC patients.

Although obesity and alterations in body composition are highly relevant in both the incidence and the outcome of EC, improving diagnosis might be as important to increase survival rates. Therefore, in **part II** of this thesis, we focussed on finding a novel diagnostic biomarker for early and accurate detection of EC. We focused on microRNA (miRNA) detection, since aberrant miRNA expression has been linked to various diseases including cancer and play an important role in the onset of obesity and obesity-related diseases. We performed a systematic review of the literature and showed a panel of miR-205, miR-200 family, miR-135b, miR-182, miR-183 and miR-223 to be promising in diagnosing EC (**Chapter 7**). This panel was further tested and validated in our own cohort in **Chapter 8**, in which we examined miRNA expression levels in Formalin-Fixed, Paraffin-Embedded (FFPE) samples of patients diagnosed with EC in our institution. We showed that the panel of miR-200a, miR-200b, miR-200c, miR-205 and miR-182 was highly accurate in distinguishing benign endometrial tissue from EC with sensitivity of 92% and specificity of 89%.

We further aimed to find a novel non-invasive diagnostic test for earlier and easier diagnosis of EC. In **Chapter 9**, we therefore focused on miRNA expression in urine. We showed miR-223 to be deregulated in urine samples of EC patients. Thus, we showed the feasibility of urinary miRNA testing as a first step in finding a non-invasive biomarker for the screening and detection of EC. In addition, we showed that several urinary miRNAs are associated with obesity. Therefore, miRNAs may be useful as biomarker for obesity and offer new information to better understand the obesity-related endometrial carcinogenesis.

Lastly, in **Chapter 10** we discuss these findings in relation to current literature and we suggest future directions for EC research. In addition, we make recommendations that can be applied to clinical practice.

Nederlandse samenvatting

Endometriumcarcinoom (EC) is de meest voorkomende gynaecologische kanker in de Westerse wereld. Zoals beschreven in **Hoofdstuk 1**, wordt EC onderverdeeld in endometrioïde endometriumcarcinomen (EEC), vaak geassocieerd met obesitas, en non-endometrioïde endometriumcarcinomen (NEEC), die ontstaan vanuit atrofisch endometrium en minder gerelateerd zijn aan obesitas. Echter, recente data laten zien dat de risicofactoren die gerelateerd zijn aan obesitas ook een rol spelen in NEEC. Desondanks zijn er nog veel onduidelijkheden wat betreft de obesitas-gerelateerde endometrium carcinogenese.

Om de invloed van obesitas op EC en uitkomstmaten verder te bestuderen, worden in **Hoofdstuk 2** en **3** van dit proefschrift de invloed van vetverdeling en spiermassa in EC beschreven. In **Hoofdstuk 2** bekijken we de vetverdeling op basis van CT-scan-metingen in relatie tot mortaliteit in hooggradige EC-patiënten. We hebben aangetoond dat een hoog visceraal vetpercentage samenhangt met een verminderde totale en ziekte-specifieke overleving in NEEC, maar niet in hooggradige EEC-tumoren. Daarnaast hebben we het verband laten zien tussen een hoog visceraal vetpercentage en comorbiditeit zoals hypertensie en diabetes mellitus (DM) bij patiënten met hooggradige EC.

Naast overgewicht hebben een toenemend aantal studies laten zien dat ook spiermassa een belangrijke rol speelt bij de gezondheidsstatus van patiënten. In **Hoofdstuk 3** hebben we gekeken naar sarcopenie, wat gedefinieerd wordt als het verlies van spiermassa, en hebben we aangetoond dat sarcopenie niet gerelateerd is aan overleving bij patiënten met hooggradige EC. Er bleek echter wel een relatie te bestaan tussen sarcopene obesitas en slechtere OS in hooggradig EEC, hoewel bij patiënten met NEEC-tumoren dit verband niet werd gevonden. Om de overlevingscijfers van patiënten met EC te verbeteren, zouden we ons niet alleen op Body Mass Index (BMI) moeten richten, maar ook aandacht moeten besteden aan spiermassa en type vetverdeling. Daarmee zouden we de counseling van deze patiënten kunnen verbeteren, door met hen de mogelijkheden van gespecialiseerde trainingsprogramma's en voedingsadviezen te bespreken.

Obesitas is een multifactorieel probleem en wordt vaker gezien bij mensen met een lagere sociaal-economische status (SES). In dit proefschrift hebben we de correlatie onderzocht tussen sociaal-economische deprivatie, BMI en overleving in EC. In **Hoofdstuk 4** worden de resultaten van een systematic review van de literatuur gepresenteerd, waaruit blijkt dat patiënten met EC een slechtere overleving hebben wanneer zij uit een laag sociaal-economisch milieu komen. Ter aanvulling hebben we een retrospectieve cohort-studie verricht in het Royal Cornwall Hospital, waarin we demonstreren dat de verschillen in overleving bij EC

met name te maken hebben met factoren zoals obesitas, gevorderd stadium bij diagnose en leeftijd (**Hoofdstuk 5**). De onafhankelijke relatie tussen obesitas en overleving was alleen significant bij patiënten met EEC. Bij patiënten met NEEC was het stadium ten tijde van de diagnose de belangrijkste prognostische factor.

Obesitas veroorzaakt een chronische inflammatie waarbij een stijging van pro-inflammatoire cytokinen en acute fase-eiwitten wordt gezien. In **Hoofdstuk 6** hebben we de invloed van hematologische parameters op overlevingscijfers in EC onderzocht, alsmede de relatie van deze parameters met obesitas en comorbiditeit (hypertensie, DM). We hebben laten zien dat anemie een onafhankelijke prognostische factor is, terwijl leukocytose met name gerelateerd is aan obesitas en de aanwezigheid van comorbiditeit. Deze parameters kunnen in patiënten met EC een belangrijke rol spelen in het voorspellen van de uitgebreidheid van de ziekte, prognose en de gezondheidsstatus.

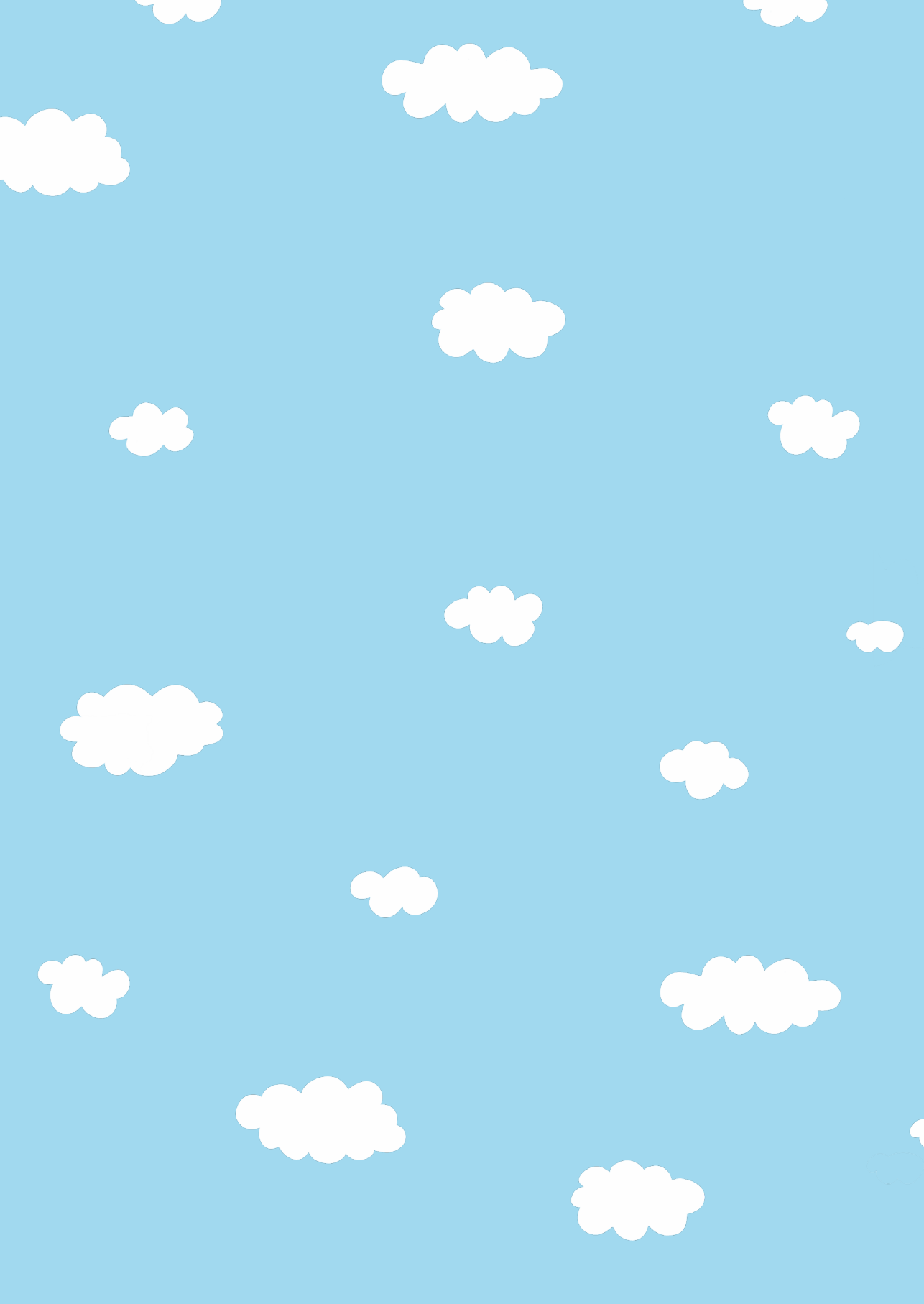
Naast het feit dat obesitas en veranderingen in lichaamssamenstelling belangrijk zijn in de incidentie en de overleving bij EC, is het vroeg ontdekken van EC misschien wel even belangrijk om de overlevingscijfers te vergroten.

In **deel II** van dit proefschrift focussen we daarom op het vinden van een nieuwe diagnostische biomarker voor vroege en accurate diagnose van EC. We hebben hierbij gekeken naar microRNA (miRNA) expressie, aangezien afwijkende miRNA expressie gezien wordt in een breed scala aan ziekten, waaronder kanker. Daarnaast speelt miRNA een belangrijke rol in het ontstaan van obesitas en obesitas-gerelateerde ziekten. In **Hoofdstuk 7** laten we middels de resultaten van een systematic review van de literatuur zien dat een panel met: miR-205, de miR-200 familie, miR-135b, miR-182, miR-183 en miR-223, veelbelovend is om de diagnose van EC te stellen. We hebben dit panel van miRNAs verder getest en gevalideerd in ons eigen cohort in **Hoofdstuk 8**. Hierin hebben we gekeken naar miRNA expressie levels in formaline-gefixeerd paraffine-ingebed weefsel van patiënten gediagnosticeerd met EC in het Royal Cornwall Hospital. Een panel van miR-200a, miR-200b, miR-200c, miR-205 en miR-182 kon accuraat benigne endometriumweefsel onderscheiden van kankerweefsel met een sensitiviteit van 92% en specificiteit van 89%.

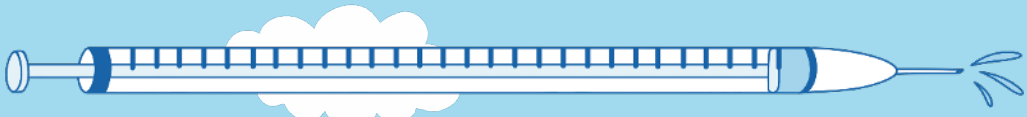
Daarnaast hebben we een nieuwe niet-invasieve methode geëxploreerd om de diagnose van EC te bespoedigen en vereenvoudigen. In **Hoofdstuk 9** hebben we laten zien dat miR-223 gedereguleerd is in urine van patiënten met EC. Daarmee hebben we gedemonstreerd dat het mogelijk is om miRNA uit urine te isoleren, als eerste stap voor het vinden van een nieuwe niet-invasieve diagnostische test om te screenen op EC. We hebben tevens aangetoond dat de expressie van meerdere miRNAs in urine afwijkend is bij vrouwen met obesitas ten opzichte van

vrouwen met een normaal BMI. MiRNAs kunnen dus niet alleen belangrijk zijn bij het diagnosticeren van EC, maar ook een biomarker zijn voor obesitas en daarmee nieuwe informatie verschaffen voor een beter begrip van de obesitas-gerelateerde endometrium carcinogenese.

In **Hoofdstuk 10** worden tenslotte de resultaten van de verschillende studies in dit proefschrift bediscussieerd en worden onze bevindingen vergeleken met de literatuur. Hierbij hebben we enkele aanbevelingen voor de klinische praktijk en toekomstig onderzoek geformuleerd.



Appendix



Dankwoord

First and foremost, I want to thank **Khadra Galaal**. Khadra, without you, this thesis would not have been possible. You have been my supervisor and mentor, but most importantly, my friend. I am grateful for all the hard work and effort you put into my PhD, despite your own busy lifestyle. I am thankful for the spare weekends you were willing to work together, sitting in your kitchen with our laptops. Not only working, but also drinking tea (herbal, I will never get used to the milky British one) and discussing life issues. I've learned to trust you and your opinions blindly, even though it might not always have felt that way. I've been stressed out about a lot of things, quite regularly, but you could always calm me down and tell me everything would be fine. You've told me ''didn't I tell you??'' over and over again. You were always joking about the fact I was bullying you, because I kept harassing you with emails, texts and phone calls. ''When are you going to look at my work Khadra???''. And you sometimes deliberately ignored me because you felt guilty you had not looked at it yet.

It has been great working with you and I'm inspired by you, your work ethic and your personality. However, this is not the end of an era. Completing this PhD and moving back to the Netherlands does not mean you're not going to hear or see me anymore and I hope we can continue working together. You're a star.

De tweede persoon die ik wil bedanken is **Ruud Bekkers**. Ruud, het enthousiasme voor de gynaecologische oncologie is misschien wel begonnen door het eerste gesprek wat wij hebben gehad in 2011 over mijn onderzoeksstage in Australië. Jaren later begon ik als ANIOS in het Rijnstate, waar ik Anke Smits leerde kennen. Alsof het zo had moeten zijn, vertelde zij over Truro en haar PhD, wat mede mogelijk was gemaakt door jou. In 2016 zijn onze paden dus weer gekruist en is het balletje heel vlot gaan rollen. Ik wil je bedanken voor je inzet en hulp de afgelopen jaren. Doordat ik in Engeland woonde, hebben we elkaar natuurlijk niet zo vaak kunnen zien, maar elke (skype) meeting was inspirerend. Ik heb bewondering voor jou als arts en onderzoeker, maar ook als persoon en je instelling in het leven. Ik hoop dat we nog vaker samen zullen werken.

Hanny Pijnenborg is de volgende die ik wil bedanken. Hanny, je bent pas later betrokken geraakt bij mijn PhD, maar in de tijd dat we samen hebben gewerkt ben je enorm waardevol geweest. Ik heb geen idee hoe je het voor elkaar krijgt om zo'n drukke baan en druk leven te hebben, en toch zo snel elke keer weer op mijn e-mails kunt reageren. Niet alleen onderzoekinhoudelijk heb ik veel aan je gehad, maar ik ben ook heel blij met de fijne, vriendelijke en open manier van communiceren die we hebben gehad en de interesse die je hebt getoond in mijn persoonlijke leven. We zullen elkaar sowieso nog tegenkomen, in Nijmegen, bij

ENITEC meetings en bij toekomstige onderzoeksprojecten.

Leon Massuger, bedankt voor de mogelijkheid en kansen die u mij heeft gegeven om dit proefschrift tot een succes te maken.

Uiteraard wil ik de leden van de manuscriptcommissie **professor dr. L.A.L.M. Kiemeney**, **professor dr. J.J. Fütterer** en **dr. H.M.J. Werner** hartelijk bedanken voor het beoordelen van mijn proefschrift en het plaatsnemen in de commissie.

Pap en mam, zonder jullie was ik nooit gekomen waar ik nu ben. Jullie hebben me altijd bijgestaan en gesteund in mijn keuzes (behalve motorrijden en het terugrijden vanuit Engeland in een oude auto waarbij last minute de accu nog vervangen moest worden en vervolgens nog de gehele rit lampjes hebben gebrand). Dat betekende wel dat jullie dochtertje voor meerdere jaren naar Engeland wilde verhuizen, maar ook hierin zijn jullie niets dan steunend geweest. Bedankt dat jullie altijd hebben geluisterd als ik weer eens gestrest was, maar ook enthousiast voor me waren als dingen liepen zoals ik wilde.

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Mam, je bent de liefste moeder die ik me kan wensen. Je bent sterker dan je denkt en samen slaan we ons door de moeilijke tijden heen die nog gaan komen.

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Milou, iemand die je in 2.5 jaar tijd 4 keer komt opzoeken in het buitenland, is toch wel even een vermelding en bedankje waard. Ik ga niet te veel woorden vuil maken want je weet wat je voor me betekent en dat is de wereld. Dank dat je bent wie je bent.

Jacqueline en Mahlet, mijn paranimfen, ook al weten jullie dat nog niet op het moment dat ik dit schrijf (wellicht ben ik een beetje laat geweest met jullie hiervoor vragen). Dank voor alle leuke dingen die we samen hebben beleefd en voor de steun in de moeilijkere tijden. Corona is een shitball maar ik heb het idee dat het ons alleen maar dichter bij elkaar heeft gebracht! Ik kan niet wachten tot we ook Mare mee kunnen nemen in onze wijn- en dansavonden (Pib doet al af en toe mee).

Anke, jij kunt natuurlijk ook niet ontbreken, want zonder jou had ik nooit van het bestaan van Truro, Khadra of het Royal Cornwall Hospital afgeweten! Des te leuker dat ik de laatste loodjes heb kunnen doen terwijl ik bij jou woonde, en in het zonnetje in jouw tuin dit heb kunnen schrijven.

Dit dankwoord wordt te lang als ik iedereen een persoonlijk stukje geef dus maar op deze manier: **Tom, Laura, Marit B, Dipti, Isha, Marit T, Nynke, Geertje, Bo, Marjolein, Maud, Myrthe, Els, Louise, Diede, Kaz, Coco, Accie in de Taxi (sorry huidige naam vond ik niet passend in een proefschrift), basketbalmaatjes, mijn NOK-collega's, de Stennies;** dankjulliewel voor alle support de afgelopen jaren, de feestjes, de kaassouffles (nee geen gekke dingen denken Stenverts), de lange wandelingen in het Ooij, de liefde van katten, de wijntjes, de spelletjesavonden, de sportactiviteiten, de stokbroodjes kruidenboter, de kaasjes, de high wines, de carnavals- en vierdaagse feestjes en alle andere dingen die ik nu vergeet.

Sophie, Daisy, Laura: thank you guys so much for making me feel at home right from the start. I have had the most amazing time living in the UK because of you guys and I will always remember the memories that we've made. I will miss dog Jasper, the cobweb palace, the long walks, the drinking, the garlic bread pizzas, offending your cheese, roast dinners, the Hubbox, Tropical Pressure Christmas parties, Sinterklaas (''sinterklaus''), being the bob, and everything else that we've shared. Ich loebe du. I like it, I love it, I'm gonna do it.

A massive thank you to everyone else that I've met while living in Truro; **Tom, Matt, Rachel, Chris, Mieke and Dorian, Dave, Anne, Dom and the entire Rebels Basketball club, Jez and the bootcampers, all my colleagues in Royal Cornwall Hospital**; thank you. You are all truly wonderful.

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List of publications

Donkers H, Fasmer K, McGrane J, Pijnenborg J, Bekkers R, Haldorsen I, Galaal K. Obesity and visceral fat: Survival impact in high-grade endometrial cancer. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 2021 Jan; 256:425-432. doi: 10.1016/j.ejogrb.2020.11.050. Epub 2020 Nov 21.

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Submitted

Donkers H, Hirschfeld M, Weiß D, Erbes T, Jaeger M, ENITEC-consortium, Pijnenborg J, Bekkers R, Galaal K. Analysing microRNA for the diagnosis of endometrial cancer: A step in the right direction?

Donkers H, Hirschfeld M, Weiß D, Erbes T, Jaeger M, ENITEC-consortium, Pijnenborg J, Bekkers R, Galaal K. Detection of microRNA in urine to identify patients with endometrial cancer: a feasibility study

Vrede S, **Donkers H**, Reijnen C, Smits A, Visser N, Geomini P, Ngo, H, van Hamont D, Pijlman B, Vos M, Snijders M, Bekkers R, Galaal K, Pijnenborg J. Abnormal pre-operative haematological parameters in endometrial cancer; reflecting tumour aggressiveness or poor health status?

Donkers H, McGrane J, Eleuteri A, Giamougiannis P, Bekkers R, Galaal K. The impact of socioeconomic deprivation on mortality in cervical cancer patients in Cornwall (England).

Donkers H, Bekkers R, Galaal K. Systematic review on Socioeconomic Deprivation and Cervical Cancer: Inequalities in Survival.

Research Data Management

This thesis is based on the results of human studies, which were conducted in accordance with the principles of the Declaration of Helsinki. All collected patient material is coded and all data were anonymised by giving each patient an individual study number.

Ethical and institutional approval was obtained fur the studies conducted in Chapter 2 and 3 in accordance with the Declaration of Helsinki and was approved by the Health Research Authority (HRA) with reference number 19/SW/0111. The CT-scan images are stored on a password encrypted hard-drive and archived in the department archive of the Royal Cornwall Hospital Trust. For Chapter 6, full ethical approval was obtained through HRA with reference number 236426. Studies conducted in Chapter 8 and 9 were approved by the HRA and Care Research Wales (HCRW) with REC reference number 19/ES/0007. The written informed consent forms are stored and archived in the department archive of the Royal Cornwall Hospital Trust. Tissue specimens were collected centrally at the department of pathology, Royal Cornwall Hospital Trust. An audit trail was incorporated to provide evidence of the activities that has altered the original data. The privacy of the participants in this study is warranted by the use of encrypted and unique individual subject codes. The code was stored separately from the study data.

Studies conducted in Chapter 4 and 7 were review articles and therefore, no ethical review board approval was needed.

The patient data for the analyses of all studies is stored on the Royal Cornwall Hospital departments' S-drive (S:\RCH\ObstGyn-Gynaeonc\Investigators file\ Database). Data will be saved for 15 years after termination of the studies. Using these patient data in future research is only possible after a renewed permission by the patient as recorded in the informed consent. The datasets analysed during these studies are available from the corresponding author on reasonable request.

Name PhD candidate: ***Hannah Donkers***	**PhD period:** ***01-08-2017 – 18-11-2020***
Department: ***Obstetrics and Gynaecology***	***Promotor:*** ***Prof. Dr. R.L.M. Bekkers***
Graduate School: ***Radboud Institute for Health Sciences***	***Co-promotor:*** ***Dr. J.M.A. Pijnenborg***

	Year(s)	ECTS
TRAINING ACTIVITIES		
Courses & Workshops		
Introduction day Radboudumc	2020	0.5
Graduate School specific introductory course	2020	0.75
BROK course	2020	1.5
Good Clinical Practice course	2017	0.5
Valid Informed Consent course	2017	0.25
Medical Statistics course	2017	1.15
Statistics course	2018	0.5
Cochrane Course Oxford	2018	1.0
Good Clinical Practice Refresher	2019	0.25
Scientific Integrity Course	2021	1.0
Symposia & congresses^		
European Network of Individual Treatment in Endometrial Cancer (ENITEC) meeting (Liverpool)^	2019	1.0
European Society of Gynaecological Oncology (ESGO) meeting (Athens)^	2019	2.0
International Gynecologic Cancer Society (IGCS) Annual Global Meeting (digital)^	2020	1.0
Other		
Bimonthly Journal Club Gynecologic Oncology	2017-2019	2.0
Monthly audit Obstetrics and Gynecology	2017-2019	4.0
TEACHING ACTIVITIES		
Lecturing		
Lecturing for trainees in Obstetrics & Gynaecology	2017-2019	0.5
Lecturing for medical students	2017-2019	0.5
TOTAL		**18.4**

^Indicate oral or poster presentation

Curriculum Vitae

Hannah Donkers werd op 11 april 1991 geboren in Gemert te Noord Brabant.
Zij volgde middelbaar onderwijs aan het Commanderij College te Gemert, waar zij in 2009 haar Gymnasium diploma behaalde en het geluk had om direct te mogen beginnen met de studie Geneeskunde aan de Radboud Universiteit Nijmegen.

Al vroeg tijdens de studie Geneeskunde werd haar interesse gewekt voor de gynaecologie en verloskunde en deze passie werd nog meer aangewakkerd tijdens haar onderzoekstage in de gynaecologische oncologie in Melbourne, Australië. Tijdens haar coschap gynaecologie in het Bernhoven ziekenhuis in Uden, wist zij het zeker: gynaecologie it is. De coschappen werden afgesloten met een tropencoschap in Tanzania en een seniorcoschap op de afdeling Gynaecologie en Verloskunde in het Rijnstate Ziekenhuis Arnhem. In 2016 haalde zij haar doctoraalexamen en startte als arts-assistent op dezelfde afdeling in het Rijnstate Ziekenhuis.

Na een jaar met veel plezier in Arnhem gewerkt te hebben, begon het te kriebelen voor een nieuw avontuur in de onderzoekswereld en in het buitenland. Al vlot ging het balletje rollen om dit avontuur in Engeland aan te gaan. In augustus 2017 is zij begonnen aan haar promotietraject in de gynaecologische oncologie in het Royal Cornwall Hospital NHS Trust in Truro, Cornwall, United Kingdom. Zij werd hierin begeleid door miss Khadra Galaal, Prof. Dr. Ruud Bekkers en Dr. Hanny Pijnenborg. Tijdens haar promotietraject heeft zij met veel plezier gewerkt als arts binnen de gynaecologische oncologie en de nodige klinische vaardigheden geleerd en op peil gehouden. Daarnaast heeft zij mogen proeven van de Engelse cultuur, binnen en buiten het ziekenhuis, en een hele fijne periode hier gehad. Na het afronden van dit proefschrift zal zij haar carrière als gynaecoloog in opleiding starten binnen het cluster Nijmegen.

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