Novel Approaches to Diagnostics for Women at High Risk for Endometrial Cancer: A Holistic Approach

by

Shalkar Adambekov

BSc Biotechnology, University College London, United Kingdom, 2009

MSc Biotechnology, L.N. Gumilyov Eurasian National University, Kazakhstan, 2012

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This dissertation was presented

by

Shalkar Adambekov

It was defended on

May 15, 2020

and approved by

Thomas J Songer, PhD, Assistant Professor, Epidemiology, Graduate School of Public Health, University of Pittsburgh

Iva Miljkovic, MD, PhD, Associate Professor, Epidemiology Graduate School of Public Health, University of Pittsburgh

Yue-Fang Chang, PhD, Research Associate Professor, Neurological Surgery, School of Medicine, University of Pittsburgh

Thesis Advisor: Faina Linkov, PhD, Assistant Professor, Epidemiology Graduate School of Public Health, University of Pittsburgh

Faina Linkov, PhD

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Shalkar Adambekov, PhD

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Abstract

Endometrial cancer (EC) is the most common cancer in US women with increasing incidence driven by epidemic of obesity and metabolic syndrome (MetS). There is an urgent need for early identification of women at high risk for EC and targeting them with effective diagnostic methods. The aim of this research work was to improve diagnostics of EC and its precursors by using holistic approach to diagnostics of EC which includes: (1) improved identification of women at high risk for EC development by identifying the most applicable MetS definition for EC; and (2) identifying factors that can be targeted to increase the success rate of Pipelle biopsy, which is the most common endometrial sampling method US. This research work demonstrated that there is a substantial diversity in MetS definitions as applied to women with EC, potentially limiting the clinical use of MetS due to inconsistencies in the research evidence. It also demonstrated that Pipelle biopsy failure rate is higher than was traditionally thought, with a number of personal and clinical factors affecting the risk of procedure failure. Holistic Model for Diagnosis of EC (HOMDEC) framework was developed based on the findings of this research work, as well as previously published research literature. Utilization of this novel holistic framework in the clinical care for women who are at EC risk has high public health significance, as it can potentially lead to reducing EC mortality due to timely, effective, and patient oriented diagnostics of EC. Further research needs to concentrate on exploring the effects of anxiety and pain during the Pipelle

procedure and subsequently working to adapt HOMDEC framework to clinical practice by collaborating with providers and hospital managers.

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1.0 Introduction

1.1 Endometrial Cancer is on the Rise

Endometrial cancer (EC) is one of the most common gynecologic malignancies in women, with incidence and mortality increasing in the developed world due to a number of factors including metabolic syndrome (MetS), obesity, ageing population, and sedentary life style[1-3]. This gynecologic cancer is expected to cause more than 65 thousand new EC cases and more than 12 thousand deaths in 2020[4]. Moreover, our group has estimated a 1.5 increase in the EC incidence by the next decade[5], which highlights the importance of improvement in diagnostic and preventive tools for this malignancy.

EC is generally divided into two subtypes based on histological findings, expression hormone receptors, and tumor grade[6]. The most prevalent subtype is Type I, which is low-grade, endometrioid, hormone-receptor positive EC, and has a good prognosis. Type I accounts for 85-90% of all ECs and is more closely associated with obesity[7]. Type II is high-grade, non-endometrioid, hormone-receptor negative, and associated with a higher risk of metastasis and mortality[6]. A recent pooled analysis of 24 Epidemiology of Endometrial Cancer Consortium studies by Setiawan suggested that BMI had a greater effect on Type I tumors than on Type II tumors: odds ratio (OR) per 2 kg/m^2 increase was 1.20 (95% CI, 1.19 to 1.21) for type I and 1.12 (95% CI, 1.09 to 1.14) for type II tumors ($P_{heterogeneity} < .0001$)[8]. A large registry based investigation demonstrated that Type I EC patients have significantly higher obesity levels compared to Type II EC patients[9]. As we are concerned with obesity associated EC, EC in this dissertation will refer to Type I disease.

1.2 Obesity is the Main Risk Factor for Endometrial Cancer

Obesity is a worldwide problem, with both developing and developed countries carrying the burden of obesity and its associated adverse health outcomes[10]. The prevalence of obesity has dramatically increased in the last few decades, reaching epidemic proportions in the US, with 36.5% of the US population living with obesity[11]. Obesity has been linked to a large number of chronic diseases such as cardiovascular disease (CVD), diabetes, and MetS. Moreover, it is a well-established modifiable risk factor for a variety of cancers. International Agency for Research on Cancer working group concluded that there was sufficient evidence to confirm the association between weight gain and increased risk for cancers of the colon, esophagus, kidney, breast, and corpus uteri[12].

Table 1. Levels of association between obesity categories and EC incidence and mortality

Weight	OR[a] of EC	RR[b] of Death from EC
Overweight (BMI 25-29.9)	1.5	1.5
Class I obesity (BMI 30–34.9)	2.5	2.5
Class II obesity (BMI 35-39.9)	4.5	2.8
Class III obesity (BMI ≥ 40)	7.1	6.3

[a]OR – odds ratios

[b]RR – relative risk

Increasing rate of obesity, especially among premenopausal women, is thought to be the primary driver of increasing EC incidence in the US population[13,14]. Levels of association between obesity categories and EC incidence and mortality are summarized in Table 1. This association follows a strong dose–response relationship, as OR of developing EC for overweight (BMI 25-29.9) was 1.5, for class I obesity (BMI 30–34.9) was 2.5, for class II obesity (BMI 35-39.9) was

4.5 OR, and for a class III obesity (BMI ≥ 40) was 7.1 when compared to normal weight populations (BMI<25)[8]. Kyrgiou et al. showed strong association between adiposity and a number of cancers, including EC, in an umbrella review of 204 meta-analyses[15]. The association between obesity and EC risk is further supported by high level of subclinical endometrial pathologies in women with obesity undergoing bariatric surgery[16,17]. Prospective studies indicate that EC risk increases 1.6-fold with each additional 5 kg/m^2 in BMI, reaching 9.1-fold higher risk at a BMI of 42 kg/m^2 [18].

One of the main hypotheses explaining association between obesity and EC is unopposed estrogen hypothesis. Endometrial proliferation is driven by the cyclic expression of estrogen by the ovaries in premenopausal women and estrogen synthesis in the peripheral tissues (mostly adipose tissues) in postmenopausal women[19]. The unopposed estrogen hypothesis of EC development posits that increased exposure to endogenous or exogenous estrogen that is not opposed by progesterone explains the relationship between obesity and EC risk[20-23].

1.3 Unopposed Estrogen Hypothesis

Estrogen is a steroid hormone that plays a critical role in the normal proliferation of endometrial tissue during the menstrual cycle. There are consistent published epidemiological data linking higher circulating estrogen levels with increased risk of EC[24,25]. Specifically, in the uterus, estrogen regulates several target genes including IGF-1 in stromal cells, progesterone receptors in endometrial cells, and several other transcription factors involved in the cell cycle and proliferation[26-28]. Increases in IGF-1 expression by stromal cells play a central role in the promotion of endometrial cell proliferation and survival in mice[27]. *In vitro* study on premenopausal

endometrial tissue shows that estrogen is opposed by progesterone, which stimulates estradiol metabolism and expression of IGF binding protein 1, with subsequent reduction in IGF-1 bioactivity in the endometrium[29]. However, in postmenopausal women, adipose tissue is the predominant source of estrogen, which results in postmenopausal women with obesity having elevated levels of estrogens compared with leaner postmenopausal women[30]. These higher estrogen levels are not counteracted by progesterone, as its production levels remain low. Thus, unopposed estrogen may be associated with excess proliferation of the endometrium and be involved in the endometrial tumor development.

Prospective investigations of postmenopausal women reported strong associations between serum levels of estradiol and risk of EC. Zeleniuch-Jacquotte et al. reported a >2-fold increased risk associated with high versus low estradiol, while sex hormone binding globulin levels were inversely associated with EC risk, after adjustment for obesity and other risk factors[31]. Consistent with these mechanistic data, use of unopposed estrogen postmenopausal hormone therapy is associated with a significantly higher risk of EC, whereas the use of the combined estrogen plus progesterone formulation appears to have a protective effect[32].

1.4 Endometrial Thickness is an Important Diagnostic Parameter for Endometrial Pathology

As an additional measure of risk for postmenopausal women, endometrial thickness greater than 4 mm may be indicative of excess estrogen stimulation that is associated with increased risk of EC and estrogen-associated endometrial pathologies, as well as breast cancer[33]. Increased endometrial thickness has been associated with obesity in previous studies[34,35]. For premenopausal

women, an endometrial thickness greater than 6 mm appears to be linked to increased risk of hyperplasia[36], which in most cases precedes endometrioid adenocarcinoma of the endometrium[37]. A recent publication highlighted the importance of evaluating thickened endometria in postmenopausal asymptomatic women due to the high risk of subclinical pathology[38].

Endometrial thickness appears to be linked to a wide range of precancerous endometrial pathologies. Previously published research suggested that endometrial thickness ranges between 5 and 20 mm based on the type of endometrial pathology[39]. Interestingly, in a pooled analysis of 4 cohort and 14 case-control studies, use of intrauterine devices reduced the odds of EC by almost 20% compared to never users[40], potentially by affecting the endometrial thickness. Since endometrial thickness in postmenopausal women appears to be increasing with advancing progression of endometrial pathology from simple hyperplasia to complex hyperplasia with atypia, this risk factor is an important variable to be investigated in studies focusing on EC risk reduction.

1.5 Endometrial Cancer and Smoking

While it is well established that smoking is a risk factor for many different cancers, smoking has a protective effect on EC development. A case-control study of 510 EC cases and 727 controls reported that compared to never smokers, smokers had 30% and 10% lower rate-ratio estimate for current smokers and former smokers, respectively[41]. These findings were further corroborated in other case-control studies with similar effect sizes[42,43]. A prospective study of 110,304 women in the US showed a significant protective effect of smoking in current and former smokers compared to never smokers[44]. Meta-analysis of ten prospective and 24 case-control studies also showed that smoking was significantly associated with a lower risk of EC in

prospective studies (RR 0.81; 95% CI 0.74 to 0.88) and case-control studies (OR 0.72; 95% CI 0.66 to 0.79)[45]. The biological mechanisms that are responsible for this association are not fully known, though a number of hypotheses suggest the role of antiestrogenic effect of smoking on circulating estrogen, relative reduction in bodyweight, and earlier menopausal age among women who smoke[46].

1.6 Metabolic Syndrome is a Risk Factor for Endometrial Cancer

MetS was initially used as a cluster of risk factors for the development of CVD and diabetes[47], however, the last decade of research demonstrated the importance of MetS as a risk factor for EC[48]. The primary component of MetS responsible for the association between MetS and EC is thought to be high BMI, which has been associated with increased risk of EC in multiple studies[49-52]. Accumulating evidence suggests that obesity is associated with chronic low-grade inflammation, contributing to systemic metabolic dysfunction, commonly associated with EC and other obesity-linked disorders. However, individual components of MetS, including central obesity[53], Type II diabetes[54-56], and a hypertensive state[57], were reported to be associated with EC.

Abdominal obesity is the most likely physiological mechanism linking MetS to EC though increase in adiposity. Many reported excess weight to be one of the strongest risk factors for EC[58], in addition to being associated with the other components of MetS[59]. Increasing adiposity leads to higher blood estrogen levels and, therefore, is considered to be a convincing causal factor for EC development based on extensive research[60]. However, waist circumference (WC) is often substituted by BMI in MetS research. These two measurements are very similar but represent

different measurements of obesity, with WC representing body fat distribution and BMI representing body mass, with both factors being associated with EC risk[53].

One other physiological factor that can explain association between EC and MetS is impaired glucose metabolism[61]. It was shown to be associated with EC in overweight women[53,62]. It is assumed that high levels of blood insulin increase the production of insulin-like growth factor 1, which results in overstimulation of proliferation of endometrial epithelium[63]. Moreover, previous research demonstrated that excess insulin was an EC risk factor independent of high BMI[64].

It was previously shown that increased amounts of dietary animal fat and cholesterol levels in blood increase the risk of EC[65], but the research on the association between blood lipids, dietary factors, and EC has not been conclusive. A positive association was found between blood triglyceride level and EC in a prospective study after 12 years of follow-up[66]. In contrast, the European Prospective Investigation into Cancer and Nutrition study did not reveal any associations between blood lipids and EC[67]. Zhang's et al. finding on positive association between EC and triglycerides and LDL-C in a Chinese case-control study[68] was partially supported by Trabert et al. in large recent case-control study in a US population[69]. Triglycerides component has changed the most among different MetS definitions, which highlights the importance of using single definition for MetS in EC patients.

The importance of hypertension in the development of cancer was outlined in subsequent Italian studies in 1989[70] and 1999[57]. Hypertension was identified as a "weak" risk factor for EC (OR= 1.6) in a Finnish study[71], supported by findings from a Swedish study where hypertension was associated with EC only in obese participants[53].

Limited number of studies have reported on the association between EC and MetS[48]. A previous meta-analysis demonstrated a significantly higher risk for a number of cancers among patients with diagnosed MetS[72], which was further supported by a meta-analysis of studies on the association between MetS and EC[48]. Existing studies did not account for the variety of MetS definitions, which is something the current research is attempting to address. Thus, association between MetS and EC is complicated by the absence of a single unified diagnostic criteria for MetS, which causes some controversy in the application of different definitions adopted in research[73].

1.7 Pipelle Biopsy is the Most Commonly Used Endometrial Sampling Method

Definitive diagnosis of EC requires collecting sufficient endometrial tissue to conduct a histological analysis. Before Pipelle endometrial biopsy became the most common procedure for endometrial sampling, dilatation & curettage (D&C) was the predominant method for obtaining endometrial samples in US[74], as it is reliable and is well tolerated by patients due to the use of anesthesia. However, D&C is performed in an operating room setting and poses inherent risks, including the use of anesthetics, infection, and perforation[75]. In addition, its high cost can be another limitation, with hospital charges ranging from $1,728 to $3,950 per procedure in the US (based on Medicare reimbursement payments)[76].

Over the past two decades, Pipelle endometrial sampling became an increasingly popular alternative to the D&C and addresses many of its limitations[77]. The main advantage of Pipelle is its lower costs, with hospital charges ranging from $318 to $644 per procedure in the US[78], and its suitability for use in outpatient settings. It also carries fewer risks and side effects[79,80]. Pipelle can

inform diagnoses for a range of endometrial pathologies including EC, endometrial hyperplasia, and atrophy[81].

The overall accuracy between the D&C and Pipelle is similar[82,83]. Demirkiran et al. performed Pipelle prior to D&C in a sample of 478 women and the outcomes showed that Pipelle and D&C were 84% concordant with each other[82]. Fothergill et al. showed that there was no statistical difference between the histological outcomes of Pipelle and D&C in a sample of 187 patients[83]. A recent study indicated that Pipelle only had a slightly lower true positive rate when compared with D&C (94% vs 96%)[80].

The main drawback to Pipelle is its higher biopsy failure rate[84]. Overall biopsy failure results from two primary reasons: (1) inability to access the uterine cavity (procedure failure) and (2) failure to obtain adequate samples for histological analyses (sample inadequacy)[77]. In our prior retrospective medical records based study, we found 23% Pipelle sampling failure rate for the general population of gynecologic patients[77]; 38.3% for severely obese (BMI≥35) bariatric surgery candidates[16]; and 42% for the postmenopausal women in a large healthcare system[77]. Hence, the potential for biopsy failure is a concern for physicians treating women at high risk for EC, and research is needed to evaluate factors influencing Pipelle success.

1.8 Pain and Anxiety can have an Effect on Pipelle Failure Rate

Our experiences at Magee-Womens Hospital clinics in Pittsburgh, along with published evidence, demonstrate that there is a significant variation in how women experience Pipelle biopsy. Some women tolerate the procedure extremely well, while other women report a high amount of pain, cramping, and discomfort. Randomized clinical trials measuring pain during the procedure

reported patients experiencing pain at level between 3.5[85] and 8[86] on a 10 point Likert scale. A number of clinical trials showed that application of local anesthesia reduces the pain[86-88] and distress[87] levels, improving overall biopsy experience. While the mitigation of pain during the biopsy was explored in clinical trials, factors associated with intensity of pain or pain itself are not explored in the current literature. Additionally, anesthetics are not commonly used during Pipelle administration in the US.

Patient mental state can have a significant impact on the choice of the procedure and how the patient experiences it. In as study of women undergoing breast biopsy, general anxiety was significantly associated with pain[89], but, to our knowledge, there are no studies reporting the effects of anxiety on Pipelle biopsy. However, studies on other invasive procedures show that anxiety can be associated with pain and discomfort during the procedure. Pontone et al. reported higher levels of pain and discomfort during the endoscopy in patients with higher levels of pre-procedure anxiety[90]. Similar results were published by a group of researchers from China who reported that pre-endoscopy anxiety was an independent predictor of severe discomfort and poor tolerance of the procedure[91]. To our knowledge, this will be the first study to explore the effects of depression, anxiety, and clinical history of mental illness on the success rate of Pipelle biopsy.

1.9 Background Summary

EC is one of the most common cancers found in women in the developed world. It is highly sensitive to obesity, which is believed to be one of the major reasons for the growing incidence of this malignancy. Despite its growing incidence, screening is currently not recommended in the general population[92]. However, the feasibility of prevention programs can be potentially different

in women with obesity and MetS, who are at higher risk of developing EC. The other important problem to be addressed in this study is Pipelle biopsy failure rate and factors associated with the failure. Despite being one of the primary diagnostic modalities, factors associated with Pipelle failure, including pain and anxiety, are not well explored in the literature. The vast majority of existing studies are based on medical records only. Identifying factors that can improve procedure success in a prospective manner will potentially help providers provide a personalized approach to each procedure based on personal characteristics of the patient in order to improve the success rate of endometrial sampling.

2.0 Paper 1: Metabolic Syndrome in Endometrial Cancer Patients: Systematic Review

2.1 Introduction

EC is the most common gynecologic cancer in the developed world, with both incidence and mortality increasing over the past decade[1-3]. Approximately 65,620 new EC cases and 12,590 deaths are expected in the US in 2020[4]. Our group previously estimated that the incidence of EC is expected to increase by 55% by 2030[5]. EC is generally divided into two subtypes based on histological findings, expression hormone receptors, and tumor grade[6]. The most prevalent subtype is Type I which is a low-grade endometroid cancer with good prognosis, commonly associated with obesity. Type II is a high grade non-endometroid cancer, associated with a higher risk of metastasis mortality[6]. High body mass index (BMI) has been associated with increased risk of EC in multiple studies for both Type I and Type II tumors[49-52,93], though the association between obesity and Type I cancer is much stronger[8]. This association follows a strong dose–response relationship with relative risk of EC increasing from 1.5 for overweight women to 7.1 for women with class 3 obesity[8]. This is further supported by a high level of subclinical endometrial pathologies in severely obese women undergoing bariatric surgery[16,17]. Accumulating evidence suggests that obesity causes chronic low-grade inflammation, contributing to systemic metabolic dysfunction, commonly associated with EC and other obesity-linked disorders. It is also possible that there is a group of EC related risk factors simultaneously affecting more than one physiological system[94]. These factors are typically unified under the umbrella of MetS.

MetS, also referred as an Insulin Resistance Syndrome or Syndrome X, is a cluster of risk factors associated with the development of CVD and diabetes, first introduced by Reaven in

1988[95]. Approximately 23% of U.S. adults were affected by this syndrome in 2010[96]. Despite the fact that MetS does not include risk factors directly causing cancer, epidemiologic, experimental and clinical studies show that there is considerable evidence suggesting that MetS is connected to the development and prognosis of several types of cancer, including EC[97,98]. There is large body of literature linking the risk of EC with individual conditions associated with MetS, including central obesity[53], type II diabetes[54-56], and a hypertensive state[57]. However, only a few studies have reported on association between EC and MetS[48]. In a previous review of 38,940 cancer cases, Esposito et. al. demonstrated a significant association between MetS and various types of cancer, including EC[72]. In the review on MetS and EC, authors confirmed the association, demonstrating that patients with MetS have higher risk of EC[48]. However, the question of prevalence of MetS in women with EC or which definition is best to use in patients at high risk for EC is still open.

Although there is a general understanding on the importance of MetS in the medical community, the clinical definition of MetS has had considerable inconsistencies in the diagnostic definitions since 1990. Numerous definitions of MetS have been used in research and clinical practice in the last decades, which resulted in research studies using one or several types of definitions, with some research groups even developing their own definitions[48,99]. To date, there are several definitions that have been commonly used: (1) The World Health Organization (WHO) was the first to present its definition in 1998[100]; (2) The European Group for the Study of Insulin Resistance (EGIR) suggested an update to the WHO definition in 1999[101]; (3) The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) in 2001[102], which was later updated by the American Heart Association and the National Heart Lung and Blood Institute[103]; (4) International Diabetes Foundation (IDF) definition was suggested in 2005[104]; and, finally, (5) Harmonized MetS guidelines was introduced in 2009 by a joint statement of the IDF,

AHA, NHLBI, World Heart Federation, International Atherosclerosis Society, and International Association for the Study of Obesity[105]. All of the definitions require at least three components (Table 2), thought the focus on particular components is different for each definition. A summary of the definitions and components of MetS is presented in Table 2.

Table 2. Definitions of metabolic syndrome

Components	WHO (1999)	EGIR (1999)	NCEP ATP III (2005)	IDF (2005)	Harmonized (2009)
Definition	Insulin resistance plus any two of the other components	insulin resistance defined (>75th percentile) any two of the other components	Any three of the components	Any three of the components	Any three of the components
Obesity	Waist/hip ratio > 0.85, or body mass index > 30 kg/m^2	waist circumference (WC) ≥ 80 cm (31.5 inches)	WC ≥88 cm (35 inches)	WC ≥80 cm for Asian origin and ≥88 cm for European origin	WC ≥80 cm for Asian origin and ≥88 cm for European origin
Dyslipidemia	Triglycerides (TG) ≥ 150 mg/dl (1.695 mmol/L) and/or high-density lipoprotein cholesterol (HDL-C) ≤ 39 mg/dl (1.0 mmol/L)	TG ≥ 150 mg/dl (2.0 mmol/L) or HDL-C < 39 mg/dl (1.0 mmol/L) or treated for dyslipidemia	TG ≥150 mg/dl (1.695 mmol/l)	TG ≥150 mg/dl (1.7 mmol/l) or history of specific treatment for this lipid abnormality	TG ≥ 150 mg/dL (1.7 mmol/l); (drug treatment for elevated triglycerides is an alternate indicator)
			HDL-C <50 mg/dl (1.3 mmol/l)	HDL-C < 50 mg/dl (1.29 mmol/l) or history of specific treatment for this lipid abnormality	HDL-C < 50 mg/dL (1.3 mmol/l) or drug treatment for reduced HDL-C
Hypertension	≥ 140/90 mmHg	≥ 140/90 mmHg or antihypertensive medication	>130/85 mmHg	systolic BP ≥130 mm Hg or diastolic BP ≥85 mm Hg or on treatment for previously diagnosed hypertension	blood pressure SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg or antihypertensive drug treatment in a patient with a

Table 2 Continued

					history of hypertension
Hyperglycemia	diabetes mellitus, impaired glucose tolerance, impaired fasting glucose or insulin resistance	impaired fasting glucose or impaired glucose tolerance, but no diabetes	Fasting plasma glucose (FPG) >110 mg/dl	FPG ≥ 100 mg/dl or previously diagnosed type 2 DM	FPG ≥100 mg/dL (5.5 mmol/l) or drug treatment of elevated glucose
Additional component	urinary albumin excretion ratio ≥ 20 µg/min or albumin/creatinine ratio ≥ 30 mg/g				

There is a substantial need to identify the most applicable definition of MetS which can be used in women at high risk of EC. Considering that women with excess weight (such as patients at high risk for EC) may delay or avoid medical care[106], ease and practicality in the application of the components of the MetS definition and diagnosis can be a deciding factor for timely treatment[107]. Moreover, single definition of MetS will help to better summarize and compare studies across the field of EC research. Therefore, the aim of this study was to review the existing literature to assess the most appropriate, practical, and comprehensive MetS definition in relation to EC, as well as to identify prevalence of MetS definitions in EC patients.

2.2 Methods

2.2.1 Search strategy

We systematically searched PubMed and Embase for articles that report associations between EC and separate components of MetS in human research participants. The key word search was performed using the following strategy: 'endometrial cancer' AND ('metabolic syndrome' OR 'syndrome x' OR 'insulin-resistance syndrome' OR 'metabolic syndrome x'). Results

were restricted to reports in English. A hierarchical approach was used to assess the relevance of studies based on the title, abstract, and the full report. The search was done in June 2018 to identify relevant articles. The PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) checklist for reporting systematic reviews and meta-analyses guideline was used in this study.

2.2.2 Study selection

We identified original studies in English, including case-control, cross-sectional, prospective/retrospective cohort, and clinical trials, which provided comparisons between women with and without EC. Non-human studies were excluded from the identified articles. Studies conducted in populations with risk factors/diseases that might predict EC independently of MetS, including conditions such as polycystic ovary syndrome or Lynch syndrome, were also excluded. Studying the effect of polycystic ovary syndrome or similar conditions might have on EC and MetS definitions is beyond scope of this review. Duplicates were removed using the method suggested by Bramer et al[108]. If a study on the same group of study participants had been published more than once, the most recent publication was used. Identified articles were further independently evaluated by two reviewers (SA and YY) for the presence of individual components common for all of the of MetS definitions: elevated waist circumference; elevated triglycerides (drug treatment for elevated triglycerides is an alternate indicator); reduced high-density lipoprotein cholesterol (HDL-C) (drug treatment for reduced HDL-C is an alternate indicator); elevated blood pressure (antihypertensive drug treatment in a patient with a history of hypertension

is an alternate indicator); and elevated fasting glucose (drug treatment of elevated glucose is an alternate indicator).

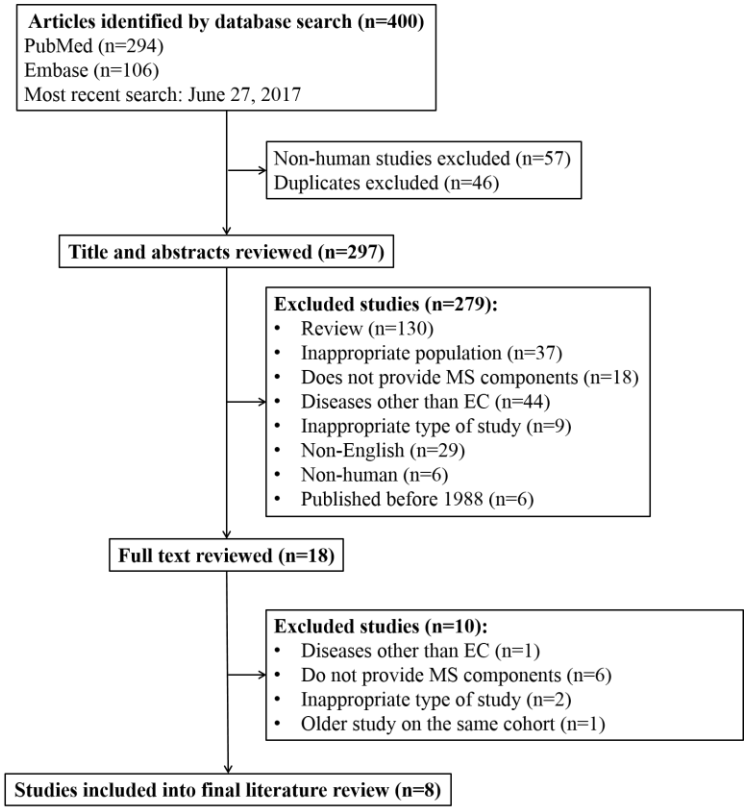

Figure 1. Flow diagram of the systematic literature search

We excluded articles that did not report measuring at least three components of MetS or articles where MetS components cannot be compared between EC and non-EC groups. Case series, case reports, conference abstracts, editorials, letters to the editor, commentaries and other types of articles not published as original research, as well as reviews, were excluded from our search.

2.2.3 Quality assessment

The Newcastle–Ottawa Scale (NOS) for non-randomized studies in meta-analyses was used to assess the quality of the selected studies[109]. This scale uses a "star system" which allocates

"stars" to case-control or cohort studies based on the selection of the study groups, the comparability of the groups, and the ascertainment of either the exposure or outcome of interest. After the independent quality review of studies by SA and YY a consensus was reached on the quality of studies and the selection of studies for the final literature review. A third reviewer (FL) was employed if there were disagreements between initial reviewers. NOS scores of ≥7 were considered as high-quality studies and of 5-6 as moderate quality, and <6 were considered low quality.

2.2.4 Data extraction

We have extracted the following information from each article that satisfied our search parameters: name of the first author, year of publication, country where the study was performed, research design, number of individuals in EC group, number of individuals in non-EC group, prevalence of MetS definitions, and each individual comparison of MetS components in EC and non-EC subgroups.

We have reviewed components for all of the definitions (WHO, EGIR, NCEP-III, IDF, and Harmonized) in each of the selected studies, and identified components that were significantly different between EC and non-EC groups. If the significantly different components fulfilled the requirements for the MetS definition, the EC subgroup in the study was marked as having MetS (Table 3).

2.2.5 Data synthesis and analysis

We assessed the feasibility of meta-analyses to evaluate the association between MetS and EC among the selected studies. A measure of heterogeneity (Q-statistic) across these studies was calculated.

2.3 Results

The results of the literature search process are presented in the flow diagram (Fig. 1). The keyword search identified 400 articles from PubMed and Embase combined, 103 of which were excluded because of non-human subjects (57), and duplicates (46). After subjecting the remaining 297 studies to title and abstract review, 271 were excluded for not meeting inclusion criteria, in particular: review (130), inappropriate population (37), did not provide MetS components (18), diseases other than EC (44), study design (9), non-English (29), non-human (6), and published prior to 1988 (6). After the title and abstract review 18 articles were selected for full text review. Out of these one was excluded for not including EC, six for not providing MetS components, two for study design, and one article was excluded because more recent publication on the study cohort was available. Eight articles published between 2007 and 2015 (six case-control, one prospective cohort, and one nested case-control) were selected for the final review[110-117]. Baseline characteristics and individual MetS components are summarized in Table 3.

Overall, this analysis included 19,739 EC cases and 387,606 controls, and included Northern American, European, Turkish, and Chinese populations. NOS quality assessment showed that three studies were of high quality[110,111,114], three studies were of moderate quality[115-117], and

two of low quality[112,113], though no studies were excluded based on quality. Four studies[110,113,115,117] specified the cancer type with all of them studying patients with both Type I and Type II tumors, while other studies did not specify cancer type. Authors used different definitions to diagnose MetS: three studies used NCEP ATP III[110,111,115] criteria; three studies used IDF[110,111,115] definition; two studies used Harmonized MetS guidelines[111,113]; three studies developed their own study definition[111,113,114], and two studies did not use any MetS definitions[112,116]. Two studies reported prevalence of NCEP ATP III and IDF definitions of MetS in their study cohorts[111,115]. The combined prevalence for NCEP ATP III definition was 17% among EC cases and 10% among controls, whereas combined prevalence of IDF definition was 6% in EC cases and 2% in controls. One study reported prevalence of Harmonized definition with 62% of cases having MetS compared to 38% controls affected by MetS[111]. Authors from three studies modified or developed their own MetS definitions. Friedenreich et al. changed waist circumference criteria for IDF from 80 to 88 cm to align more closely with the definition of abdominal obesity used for North American Caucasian populations[111]. Rosato et al. used the

Table 3. The common definitions of metabolic syndrome in identified studies

Author (year)	Country	Research design	Number of women		Predominant Race/ Ethnicity	Age	EC type	Score	MetS definitions					
			cases	controls					WHO (1999)	EGIR (1999)	NCEP ATP III (2005)	IDF (2005)	Harmonized (2009)	Study definition
Trabert et al. (2015)	USA	C-C	16,323	100,751	Caucasian, Black, Asian, Hispanic	≥65	I and II	Moderate	No	No	Yes	Yes	Yes	
Friedenreich et al. (2011)	Canada	C-C	515	962	Caucasian	30-79	NA	High	No	No	No	Yes	Yes	Yes
Rosato et al. (2011)	Italy	C-C	454	798	NA	18-79	I and II	Low	No	No	No	Yes	Yes	Yes
Zhang et al. (2010)	China	C-C	942	1721	Asian	NA*	I and II	Moderate	No	No	Yes	Yes	Yes	
Cust et al. (2007)	Denmark, France, Germany, Greece, Italy, The Netherlands, Spain, and the UK	Nested C-C	284	546	NA	47.0-71.0	I and II	High	Yes	No	Yes	Yes	Yes	
Stocks et al. (2015)	Norway, Sweden and Austria	Prospective cohort	969	282,434	NA	44.1	NA	High	Yes	No	No	No	No	Yes
Avcioglu et al. (2015)	Turkey	Case-control	46	44	NA	56.1	NA	Low	No	No	No	No	No	
Zhan et al. (2013)	China	Case-control	206	350	Asian	53.4	NA	Moderate	No	No	No	No	No	

*NA - data not available

combination of the following criteria to define MetS: (1) type 2 diabetes, (2) history of drug-treated hypertension, (3) history of a clinical diagnosis or drug-treated hyperlipidemia, and (4) abdominal obesity[113]. Stocks et al. constructed a score for the MetS by adding the individual z scores of the variables BMI, mid blood pressure, glucose, cholesterol, and triglycerides[114]. The EGIR definition was not identified in any of the selected studies. The potential confounding factors addressed in the statistical modeling varied across the studies: BMI in eight studies[110-117], age in seven studies[110-116], race/ethnicity in one study[115], education in one study[113], age of menarche in two studies[110,113], menopausal status in five studies[110,111,113,116,117], parity in five studies[110-113,116], hormone therapy use in four studies[110,111,113,116], oral contraceptives use in two studies[110,113], smoking in three studies[114-116], and alcohol consumption in one study[116]. Additional searches have not identified additional articles of interest.

We have found that IDF (63% of studies) and Harmonized (63% of studies) definitions can be most commonly identified in EC patients across all selected studies (Table 3). The NCEP ATP III definition was identified in 38% of studies, whereas the WHO definition was identified in only 25% of the studies. There were no studies that corresponded to the EGIR definition. Studies also varied in the amount of clinical data, which can be used to identify MetS. Only one (13%) out of 8 studies[110] provided sufficient data to diagnose MetS syndrome according to four definitions (WHO, NCEP ATP III, IDF, and Harmonized). Three studies (38%)[110,115,117] provided sufficient data to diagnose MetS according to three definitions (NCEP ATP III, IDF, and Harmonized).

Attempted meta-analysis of association between EC and each definition of MetS showed that there were high levels of heterogeneity among studies which limits the interpretation of the results. The heterogeneity was 83.4%, 46.5%, and 65.7% for IDF, Harmonized, and NCEP ATP

III definitions respectively. Therefore, no further meta-analysis was attempted, and further discussions were focused on the comprehensive systematic review.

2.4 Discussion

Our literature review shows that there is a large variability in the MetS definitions used for EC research. Almost 40% of the studies have employed their own definitions developed by the study authors, whereas the most commonly used definitions – IDF and Harmonized, were used in five studies. The prevalence of MetS in EC patients also varied based on the definitions ranging from 6% for IDF to 62% for Harmonized. We have also found that less than half of studies provided sufficient information for the diagnosis of MetS using the majority of definitions.

MetS was initially used as a cluster of risk factors for CVD and diabetes[47], however, the last decade of research demonstrated the importance of MetS as risk factor for EC[48]. The association between MetS and EC is complicated by the absence of a single unified diagnostic criteria for MetS, which causes controversy in the application of different definitions adopted in research[73]. In this review, we encountered seven different definitions (4 developed by professional organizations and 3 developed by the study authors), which vary in their approach to component cutoffs or emphasis of particular components[69]. In this study, we found that all the definitions except the EGIR can be consistently identified in EC patients from a variety ethnic backgrounds, though the prevalence of MetS was different based on the definition used.

The most common definitions we identified in selected studies were IDF and Harmonized, which can be explained by less stringent requirements in their definitions compared to WHO. WHO definition has insulin resistance as an obligatory component. EGIR requires insulin

resistance to be >75th percentile as an obligatory component. Data on insulin resistance were not routinely reported in the reviewed studies, which limited applicability of WHO and EGIR definitions. This was especially important in the case of EGIR definition, as none of the studies reported percentiles for insulin resistance. NCEP ATP III requires results from blood work and body measurements, which were reported more often, resulting in a higher applicability compared to WHO and EGIR definitions. In contrast, both IDF and Harmonized "relax" the requirements by introducing history of disease in the absence of bloodwork results, and Harmonized definition goes even further by suggesting history of disease or treatment as sufficient requirement. This makes both definitions the most applicable, which significantly improves the ease of identifying MetS based on a single visit from a patient. Considering that overweight and obese patients are likely to delay and avoid medical care[106], arriving at a diagnosis as quickly as possible with fewer clinical tests becomes critically important for MetS patients. This is particularly important considering that these patients are less likely to keep future appointments, leading to a reduced number of options for timely identification and treatment of MetS. Therefore, patients with EC or at risk of EC might benefit from a less stringent definitions of MetS, which we have showed to be IDF and Harmonized. Moreover, consistent use of single definition (IDF or Harmonized) might reduce the discrepancies between research studies in the area of EC, and provide better insight on the association between MetS and EC.

Another beneficial aspect of IDF and Harmonized definition is that both definitions adjust for race/ethnicity in defining central obesity. A recent study showed that the prevalence of Mets is dependent on both sex and ethnicity[118]. In addition, there are racial discrepancies related to EC morbidity and mortality. EC mortality is disproportionately higher in African American women, with 2.5 times higher rate of death compared to white counterparts[119]. The racial disparity is

highlighted by discrepancies in EC related mortality with 64% five year survival rate in African American women compared to 86% survival rate in white women[120]. It is also interesting to note that study by Trabert et al. was the only one that measured and adjusted for racial/ethnic background, however, the effect of race/ethnicity on the association between EC and MetS was not separately reported[115]. Other studies were performed on racially homogenous populations or did not report race/ethnicity background at all. With the development of definitions of MetS the role of race/ethnicity has become more important with IDF and Harmonized definitions adjusting for racial/ethnical background in defining central obesity[104,105], however, there are currently no MetS definitions specific to racial or ethnic background adjusting for other MetS components. The role of race/ethnicity is not well studied within the context of association between EC and MetS and requires further investigations and comparisons of populations with otherwise similar risk factors.

There are several strengths to this study. First, to our knowledge, this is the first review to address the discrepancies in MetS definitions in the context of EC, or any other cancer. Second, we used robust methodology to identify, access, and extract data consistent with systematic reviews from the existing literature. Third, we have used peer reviewed publications, which supports our aim of identifying the most appropriate MetS definition for EC patients.

There are several limitations to our study. One limitation is that we did not include gray literature, i.e. sources outside of commercial scientific publishing, which could have resulted in some publication bias in this study. Only half of the studies provided information on the type (Type I or Type II) of EC in the studied population, which limits our understanding of pathophysiological processes between MetS and EC. Based on the current literature, Type I EC is believed to be more strongly associated with MetS compared to Type II EC, as there is well reported association

between obesity and Type I EC[8]. However, pathophysiological link between Type II EC and MetS is still underinvestigated. Moreover, considering that different definitions of MetS focus on different components, the strength of association between MetS and EC types might vary based on MetS definition used, and requires further investigation in future studies. Another limitation is that we could not conduct meta-analyses due inconsistent use of MetS definitions as well as large heterogeneity in the selected studies. This could be explained by a small number of studies that have been included into this review, which might suggest that we had strict criteria. However, this is consistent with previously published meta-analysis, where 6 studies were selected for the final review[48]. Finally, the American Heart Association guidelines have changed the definition of hypertension from 140/90 to 130/80[121], which can potentially have a large impact on the prevalence of Mets in general population if new guidelines will be incorporated into MetS definitions. Future studies are needed to explore the effect of this change on the association between MetS and EC.

2.5 Conclusions

We have reviewed current research literature on the association between EC and MetS. Our findings support the notion that EC and MetS are connected, and MetS can be used to describe EC patients when compared to non-EC individuals. Moreover, we showed that IDF and Harmonized definitions were most applicable MetS definitions in EC patients. It is not yet clear if the discrepancy between MetS definitions is impacting other fields of cancer research, but the conclusions from this review are applicable to specifically MetS and EC research. While obesity/waist circumference being the most important MetS component in regards of EC, research

on other malignancies or diseases might prioritize different components of MetS definitions. As a result, due to comparatively small number of studies looking at MetS and EC, future research should concentrate on assessing the prevalence of MetS definitions in EC patients from large cohort studies. The importance of comprehensive MetS was also discussed in this review. We argue the need to consistently use a single well established MetS definition (IDF or Harmonized) in order to decrease the discrepancy across future studies, improve understanding of the evidence regarding the association between MetS and EC, and increase practical application of MetS in patient care.

Significance. There is a substantial need to identify the most practical definition of MetS which can be applied to women at high risk of EC. Considering that women with excess weight (such as patients at high risk for EC) may delay or avoid medical care[106], ease and practicality in the application of the components of the MetS definition and diagnosis can be a deciding factor for timely intervention / treatment[107].

Novelty. This is the first study to look at differences in MetS definitions in the context of EC, malignancy most sensitive to obesity

Aim. The aim of this study was to review the existing literature to assess the most appropriate and comprehensive MetS definition in relation to EC, as well as to identify prevalence of MetS in EC patients.

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3.0 Paper 2: Patient and Provider Factors Associated with Endometrial Pipelle Sampling Failure

3.1 Introduction

EC is the most common gynecologic malignancy in the developed world[122], with the highest incidence in the US and Canada[3]. Endometrial biopsy plays a significant role in early cancer diagnosis, preoperative assessment, and treatment planning[123,124], with Pipelle biopsy emerging as the most common method for sampling endometrial tissue in patients with suspected EC[125,126]. Pipelle sampling is a cost-effective[80] procedure and has similar sampling adequacy and histopathological results as dilation and curettage (D&C)[127]. In contrast with D&C, Pipelle biopsies are better tolerated and can be easily performed in an out-patient setting[128]. The increasing incidence of EC[5] highlights the importance of Pipelle biopsy for timely cancer diagnosis.

Despite the wide utilization of Pipelle biopsy for diagnosis of uterine pathologies, few studies have assessed the rate of sampling failure and the factors associated with the failure of Pipelle sampling procedures. A meta-analysis by Dijkhuizen et al. in 2000 found a 10.4% sample size-weighted failure rate across 15 studies that used Pipelle sampling[125], whereas a systematic review by Clark et al. in 2002 reported only an 8% failure rate among 7 studies[129]. However, individual studies over the years have reported up to 33% Pipelle sampling failure rates depending on the study characteristics and the participant inclusion criteria[128,130-134]. In a previous research study conducted by our group, Pipelle biopsy sampling had a sampling failure rate of 38% with severely obese (BMI≥35) bariatric surgery candidates[16]. Primary reasons for biopsy failure

reported in the literature are inability to access the uterine cavity or an insufficient amount of tissue collected for histological analysis[134].

Therefore, the aim of the present study was to fill an important gap in the literature by examining factors associated with the rate of failure in a sample of women who underwent Pipelle biopsy in a large healthcare system. Elucidating the factors associated with increased risk of biopsy sampling failure is important, as this information can potentially provide clinicians with additional tools to identify appropriate candidates for outpatient biopsy and to consider using alternative diagnostic options for women with high risk of failure.

3.2 Methods

After approval from the University of Pittsburgh Institutional Review, a consecutive sample of 201 patient records was selected for women who underwent Pipelle biopsy procedures for suspected uterine pathology in a large healthcare system over a period of 6 months (January - June 2013). Patient records were identified through CoPath, a pathology information system, using keywords “Pipelle”, “Endometrial” and “biopsy”, and were accessed through the UPMC Center for Assistance in Research eRecord team, per UPMC policy. Based on literature review and clinical experience, the following information was obtained from a medical records review: history of prior biopsy success/failure, age group (categorical: 22-54 and ≥55), body mass index (BMI) group (categorical: normal weight (<24.9), overweight (25.0–29.9), and obese (≥30.0)), history of smoking, history of hormone use, history of sexually transmitted diseases, gravidity, parity, indication for current biopsy, reason for current biopsy failure, and the type of the healthcare provider performing the Pipelle procedure (physician vs. other (Certified Registered Nurse

Practitioner or Physician Assistant)). We have used STRAW+10 staging system which defines the reproductive stages in a woman's life from premenopause to the late postmenopausal period to approximate age criteria for the early and late menopausal transition[135]. In accordance with the STRAW+10 guidelines women in this study were dichotomised into postmenopausal ≥55 years (n=64 (31.84%)) and pre-menopausal 22-54 years (n=137 (68.16%)) groups based on age as proxy variable, and, roughly corresponding to atrophic endometrium and non-atrophic endometrium respectively.

The data abstracted from the medical records is assumed to be reliable for all information collected within UPMC facilities and all procedures conducted at the UPMC facilities. Over 60% of women residing in Allegheny County use UPMC facilities for their medical care, with a large percentage of genecology patients solely relying on UPMC facilities for their care. While information on personal factors like gravidity, parity, STDs, etc., is not necessary collected at the biopsy visit, it is confirmed at each consultation visit and/or medical encounter leading to the biopsy appointment.

Biopsy failure was defined as a dichotomous outcome (biopsy failure/success) based on the following definition: 1) the clinician was unable to introduce the Pipelle curette into the uterine cavity; or 2) an insufficient amount of tissue was obtained during Pipelle biopsy procedure for histological evaluation. This definition was utilized for current biopsy attempt and for the history of previous biopsy failure. All collected endometrial tissue samples were examined by pathologists who determined sample adequacy for histological diagnosis and provided a pathology report.

Descriptive statistics were used for initial data analyses. In our models, we assessed each one of the above factors using univariate logistic regression to determine if they were significantly

associated with sampling failure. To control for possible confounding factors we used multivariable logistic models adjusting for age and BMI, as these factors have been associated with biopsy success/failure in the literature and in our clinical practice[16,136].

Variables that were significant in the multivariable models and were reported as factors influencing sampling success in the clinical practice of our healthcare system were then tested for interactions to identify any effect modification in our logistic regression models, while adjusting for BMI and age. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC) with α level set at <0.05 (two-sided). Missing values were excluded from the analysis. Statistically insignificant risk factors (p-value>0.05) were excluded from the final model.

3.3 Results

The characteristics of the study participants are presented descriptively in Table 4. Briefly, the majority were white, between the ages of 22-54, and overweight or obese. The Pipelle sampling failure rate among physicians was 39 out of 185 (21.08%) compared to 7 out of 16 (43.75%) failure rate among non-physician providers.

Univariate regression (Table 5) demonstrated that older age (OR 4.15, 95% CI 2.04-8.45, P<0.001), history of failed Pipelle biopsy (OR 15.01, 95% CI 2.65-84.94, P=0.002), postmenopausal bleeding as an indication (OR 3.68, 95% CI 1.34-10.09, P=0.015), and non-physician provider type (OR 5.37, 95% CI 1.69-17.07, P=0.004) were significant predictors of

Table 4. Characteristics of the study population by Pipelle biopsy sampling success vs. failure

Demographic and personal characteristics of study participants	N (%)	Pipelle Biopsy Sampling	
		Failure	Success
		46 (22.89%)	155 (77.11%)
Race (N=201)			
White	170 (84.58%)	40 (23.53%)	130 (76.47%)
Non-white	31 (15.42%)	6 (19.35%)	25 (80.65%)
Age group (N=201)			
22-54	137 (68.16%)	19 (13.87%)	118 (86.13%)
≥55	64 (31.84%)	27 (42.19%)	37 (57.81%)
BMI group (N=200)			
Normal (≤24.9)	57 (28.50%)	9 (15.79%)	48 (84.21%)
Overweight (25.0–29.9)	55 (27.50%)	12 (21.82%)	43 (78.18%)
Obese (30.0+)	88 (44.00%)	24 (27.27%)	64 (72.73%)
Gravida (N=197)			
None	39 (19.80%)	9 (23.08%)	30 (76.92%)
1-2 pregnancies	83 (42.13%)	23 (27.71%)	60 (72.29%)
3+ pregnancies	75 (38.07%)	12 (16.00%)	63 (84.00%)
Parity (N=197)			
None	51 (25.89%)	11 (21.57%)	40 (78.43%)
1-2 children	93 (47.21%)	22 (23.66%)	71 (76.34%)
3+ children	53 (26.90%)	11 (20.75%)	42 (79.25%)
Smoking history (N=198)			
Never	128 (64.65%)	33 (25.78%)	95 (74.22%)
Ever	70 (35.35%)	11 (15.71%)	59 (84.29%)
History of hormone use (N=194)			
Never	180 (92.78%)	39 (21.67%)	141 (78.33%)
Ever	14 (7.22%)	5 (35.71%)	9 (64.29%)
History of Sexually Transmitted Diseases (N=197)			
Never	180 (91.37%)	41 (22.78%)	139 (77.22%)
Ever	17 (8.63%)	3 (17.65%)	14 (82.35%)
History of prior biopsy success (N=199)			
None	152 (76.38%)	30 (19.74%)	122 (80.26%)
Yes	47 (23.62%)	15 (31.91%)	32 (68.09%)
History of prior biopsy failure (N=199)			
None	191 (95.98%)	39 (20.42%)	152 (79.58%)
Yes	8 (4.02%)	6 (75.00%)	2 (25.00%)
Indication for reference biopsy (N=201)			
Abnormal Pap smear	29 (14.43%)	6 (20.69%)	23 (79.31%)
Excessive bleeding or irregular bleeding	104 (51.74%)	12 (11.54%)	92 (88.46%)
Postmenopausal bleeding	57 (28.36%)	26 (45.61%)	31 (54.39%)
Other	11 (5.47%)	2 (18.18%)	9 (81.82%)
Type of biopsy failure (N=46)			
Unable to access endometrium	8 (17.39%)	8 (17.39%)	NA*
Inadequate sample	37 (80.43%)	37 (80.43%)	NA
Unknown	1 (2.18%)	1 (2.18%)	NA
Type of provider (N=201)			

Table 4 Continued

Physician	185 (92.04%)	39 (21.08%)	146 (78.92%)
Other	16 (7.96%)	7 (43.75%)	9 (56.25%)

*NA, not applicable

sampling failure of Pipelle biopsy. Multivariable logistic regression modelling demonstrated that a history of prior biopsy failure (OR 23.87, 95% CI 3.76-151.61, P<0.001), an indication for biopsy of postmenopausal bleeding (OR 7.41, 95% CI 2.27-24.14, P=0.002), and provider type (OR 9.15, 95% CI 2.49-33.69, P=0.001) were significantly associated with a higher risk of having a failed Pipelle biopsy, while age group was no longer significant (Table 5). Hormone use was not significantly associated with biopsy failure. While BMI was not a statistically significant predictor of Pipelle sampling failure, it is important to point out that women who were obese had a higher

Table 5. Univariate and multivariable logistic regression analysis of risk factors associated with Pipelle biopsy sampling failure adjusting for age and BMI.

Risk factor	Univariate model		Multivariable model	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Indication				
Abnormal Pap smear	1.65 (0.53, 5.13)	0.963	2.03 (0.53, 7.80)	0.811
Other	1.13 (0.19, 6.72)	0.568	1.79 (0.25, 12.62)	0.722
Postmenopausal bleeding (reference for all indications is excessive bleeding or irregular bleeding)	3.68 (1.34, 10.09)	0.015	7.41 (2.27, 24.14)	0.002
Age group[a]				
≥55 (ref. 22-54)	4.15 (2.04,8.45)	<0.001	1.95 (0.72, 5.30)	0.193
BMI group[b]				
Obese (ref. normal)	1.50 (0.61-3.67)	0.377	1.82 (0.67, 4.90)	0.243
Overweight (ref. normal)	1.42 (0.53-3.84)	0.489	1.28 (0.42, 3.94)	0.917
History of prior biopsy failure				
Yes (ref. none)	15.01 (2.65, 84.94)	0.002	23.87 (3.76, 151.61)	<0.001
Type of provider				
Nonphysician (ref. Physician)	5.37 (1.69, 17.07)	0.004	9.15 (2.49, 33.69)	0.001

[a]Adjusting for BMI only
[b]Adjusting for Age only

percentage of Pipelle biopsy failure 24 (27.27%) compared to women of normal weight 9 (16.67%). We tested interaction terms in our multivariable models; however, no significant interactions were observed.

3.4 Discussion

Among the women who underwent Pipelle biopsy procedures at a major healthcare system, a consecutive sample of 201 patients demonstrated an approximately 23% sampling failure rate for the Pipelle biopsy, which is within the range of failure rates reported in previously published literature. We found that ≥55 age group, postmenopausal bleeding as indication for sampling, history of prior biopsy failure, and type of provider are important factors that were associated with Pipelle biopsy sampling failure.

Factors influencing sampling failure rates for Pipelle biopsies have rarely been investigated. Gordon & Westgate suggested that Pipelle biopsy sampling failure results predominantly from the insufficiency of collected samples for histological diagnosis, whereas a small proportion of failures can be attributed to barriers preventing physical access to the endometrial cavity[134]. McCluggage suggested that patients' histories, including menopausal status and hormone use, are important in determining the success of Pipelle biopsy[123]. In a recent report, Ewies et al. suggested that factors including the provider's lack of experience with endometrial biopsies, atrophic endometrium, patients' pain intolerance, and the procedure type could be responsible for the inadequacy of the biopsy sample[137]. Farrell et al. suggested that further investigation is necessary for women with inadequate Pipelle sampling, since 20% of women with

'insufficient' samples were found to have uterine pathologies on second investigation, with 14% of those having evidence of malignancy[138].

We found that patient personal characteristics including age group, a history of prior biopsy failure, a biopsy indication of postmenopausal bleeding, and the biopsy being performed by a non-physician were statistically significant individual predictors of Pipelle sampling failure, whereas in the multivariable model the effect of age group was attenuated by the presence of postmenopausal bleeding. Our findings that older age group and postmenopausal bleeding as a biopsy indication are predictors of Pipelle biopsy sampling failure corroborate previously published literature[2,136]. Postmenopausal age[135,139] has been reported to be associated with a higher likelihood of biopsy failure due to postmenopausal atrophic thinning of endometrium which results in less tissue available for sampling[140] and uterine cavity obstruction. Since EC is found approximately in 10% of all cases of postmenopausal bleeding, failure to collect sufficient samples in these patients can result in missing a significant potentially life threatening pathology[141]. Two significant factors predictive of Pipelle sampling failure reported in this paper, the history of a prior failed Pipelle biopsy and type of biopsy provider, are novel findings. It should be noted that for individual patient's other factors may have also contributed, including extreme obesity, low pain tolerance, or anatomic distortion; these warrant additional research. Though we have shown that non-physician provider type is associated with higher failure rate of Pipelle biopsy, we should interpret these results with caution because of the limited representation of non-physician providers in our sample (~8%). The Pipelle biopsy failure rate for physicians was 21% in our sample, which we consider to be a reasonable failure rate based on our clinical experience and review of the literature. We suggest that biopsies should be attempted by physicians if the general failure rate becomes notably higher than one out of five.

Strengths of this study included our ability to access records from a large healthcare system and collect a range of potential predictor variables from medical records. Another strength is that data on potential predictors were collected prior to the data on sampling failures, which allowed us to provide better exposure-outcome association. We believe that our results are generalizable to the other large healthcare systems, as our participants were geographically and ethnically diverse and came from multiple clinics within a large healthcare system.

The major limitation of this study is that our biopsy failures were originally identified through a pathology software system (CoPath) using a key word search and not by accessing patients' individual medical histories. Another limitation was that we were not able to collect information on sonographic endometrial thickness, which was reported to be significant predictor of insufficient endometrial sample[140]. Overall, our method of data collection did not allow us to capture all of the patients for whom Pipelle sampling previously failed, or to tease out provider level (primary vs. tertiary provider) for whom it failed.

Future studies should focus on prospectively collecting data from larger samples of patients to identify the failure rate across different clinical venues, and collection of more information that may predict which patients are most likely to have a successful office visit endometrial tissue sampling procedures and not require high cost endometrial sampling conducted in the OR to obtain sufficient tissue for a successful biopsy. Additionally, the development of novel protocols to reduce Pipelle biopsy sampling failures (e.g., premedication) should be investigated to improve the value of care and improve minimally invasive EC screening.

3.5 Conclusions

The present study provides novel findings that may potentially help clinicians to identify patients at high risk for Pipelle biopsy failure, including previous Pipelle biopsy failures and postmenopausal bleeding as a biopsy indicator. However, prospective research studies with real time data collection are needed to determine the mechanisms underlying the increased risk of biopsy failure associated with factors identified in this study and to explore possible patient-reported factors that may be important (e.g., anxiety).

Significance. Elucidating the factors associated with increased risk of biopsy sampling failure is important, as this information can potentially provide clinicians with additional tools to identify appropriate candidates for outpatient biopsy and to consider using alternative diagnostic options for women with high risk of failure.

Novelty. This is one of the first studies to identify factors associated with Pipelle biopsy failure

Aim. The aim of the present study was to fill an important gap in the literature by examining factors associated with the rate of failure in a sample of women who underwent Pipelle biopsy in a large healthcare system.

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4.0 Paper 3: Prospective Investigation of Factors Associated with Outpatient Endometrial Biopsy Failure

4.1 Introduction

Pipelle endometrial biopsy is one the most widely employed methods for clinical evaluation of postmenopausal bleeding. Postmenopausal bleeding is a common gynecological problem affecting up to 10% of postmenopausal women and always warrants further clinical follow-up[142]. Postmenopausal bleeding has many potential etiologies, but EC and endometrial hyperplasia are the most concerning potential causes and must be ruled out. EC is the most common gynecologic malignancy in the developed world, and the incidence of EC is projected to increase significantly in coming decades[5,77,122,143]. Early surgical intervention is curative, making early detection through biopsies a crucial component of the management of EC[77,123,126].

Prior to widespread introduction of Pipelle biopsy in the US, the gold standard approach for obtaining endometrial tissue for diagnosing EC has been D&C performed in an operating room[144]. Due to costs associated with the procedure (e.g., personnel, equipment, use of anesthesia), D&C is expensive and time-consuming when compared to office-based methods of endometrial sampling, such as Pipelle biopsy performed with suction curette[145-147]. In addition, D&C is associated with higher rates of complications, including those directly related to the procedure (e.g., infection, perforation) and those related to the risks of anesthesia[148]. In contrast, Pipelle biopsy is a minimally invasive outpatient endometrial biopsy procedure that can be performed in a physician's office[123]. Pipelle biopsy is cost-effective, has a similar level of accuracy as D&C, and is associated with lower risk of perforation and hemorrhage[125,128,144,149].

While cost and complications are of concern for D&C, Pipelle endometrial sampling has been criticized for having higher rates of failures compared to D&C[134], mainly due to procedure failure or sample inadequacy. Historically, Pipelle failure rates have been considered to be approximately 10% for procedure failure and another 10% for sample inadequacy, resulting in 20% overall biopsy failure[150]. Our group, however, previously found an overall failure rate of 38% in obese women[16] and a 23% overall failure rate in a more general clinical sample using medical records review[77]. Factors including biopsy indication, prior history of biopsy failure, type of provider, and endometrial thickness have been linked to overall Pipelle biopsy failure in published literature[77,140]. However, the majority of prior publications on Pipelle biopsy failure, including our own prior research[77], were based solely on retrospective reviews of electronic health records. Use of electronic health records as the sole source of data regarding Pipelle biopsy failure potentially limits our understanding of factors associated with procedure failure since not all Pipelle biopsy attempts are recorded (e.g., due to billing concerns) during routine clinical care. Because of the potential for underestimation of procedure failure rates in medical records, there is a need for prospective studies of Pipelle biopsy in routine clinical practice.

Pain is an important factor for Pipelle biopsy, and there is a large variation between how much pain women experience during this procedure[151]. There are a number of studies addressing the pain issue for Pipelle biopsy by administering a variety of anesthetic agents prior to the procedure. Dogan et al. reports a randomized clinical trial assessing effectiveness of naproxen only, lidocaine only, and lidocaine plus naproxen compared to placebo[87]. The results of the study show that only lidocaine plus naproxen group experience significantly less pain compared to controls. The levels of distress were also lower in the lidocaine plus naproxen group[87]. Use of anesthetics to reduce pain during the Pipelle biopsy was also corroborated by trials assessing the

effect of intrauterine lidocaine[152] and lidocaine and levobupivacaine[88] on reducing the pain during the procedure. However, though all of the studies agree that women on average experience significant pain during the Pipelle biopsy, the effect of pain on success rate of biopsies is not frequently addressed in these studies. In a randomized study comparing Pipelle and Novak biopsies, Pipelle showed significantly lower mean pain level (3.21) but also had higher failure rate of 12.8%[151]. In another study, Kosus et al. reports two times higher biopsy failure rate (20%) in control group compared to lidocaine group[88]. It is worth noting that anesthetic agents are rarely used during Pipelle biopsy in the US. Therefore, there is a need to study the effects of pain on success rate of Pipelle biopsy.

To our knowledge, there are no published studies on the association of anxiety and tolerance/failure rate of Pipelle biopsy. However, studies on other sampling procedures showed that anxiety and distress might affect the biopsy experience and pain during the procedure. In a sample of 50 image-guided breast biopsy patients, worry about the procedure prior to the biopsy was significantly related to both pain and physical discomfort, whereas general anxiety was significantly associated with pain, but not to physical discomfort[89]. Similarly, in patients undergoing esophagogastroduodenoscopy, pre-endoscopy anxiety was associated with increased risk of severe discomfort, showing a linear association between pre-endoscopy anxiety and procedure tolerance[91]. The area of anxiety and procedure tolerance is very limited for Pipelle biopsy and sampling procedures in general, highlighting the novelty of our research study.

Failed Pipelle biopsies pose a diagnostic dilemma, since repeat Pipelle sampling potentially leads to additional patient burden, while switching to a biopsy by D&C is associated with additional surgical and anesthesia risks[147], as well as escalating costs. However, foregoing further diagnostic evaluation increases the risk of missing malignancy[138]. Few previous studies have been

specifically designed to prospectively capture the incidences of procedure failure and sample inadequacy, as well as to examine risk factors for each type independently[153]. In this study, we aimed to close this gap in the literature to help better identify patients at higher risk of Pipelle biopsy failure and potentially provide guidance for future interventions.

4.2 Methods

4.2.1 Setting and recruitment

This prospective cohort study was approved by the University of Pittsburgh Institutional Review Board (PRO17030492), and signed informed consent was obtained from all participants. Study participants (N=124) were women for whom Pipelle endometrial biopsy procedures were attempted at the Midlife Health Center (primary care) or Gynecologic Specialty clinic at Magee-Womens Hospital of UPMC, Pittsburgh, PA, between August 2017 and June 2019. All attempted biopsies were performed by OB/GYN physicians. Women for whom pathology diagnoses were not ultimately obtained (N=3) were excluded from the study, which yielded a final sample of 121 patients. Data on biopsy indication and success were obtained through provider questionnaires (e.g., procedure failure/success, reasons for biopsy failure) administered immediately after the procedure was completed. Procedure failure was extracted was defined as provider responding "No" to the "For the Pipelle attempted today, were you successful in obtaining a sample?" question from the provider questionnaire. The inadequacy of acquired biopsy samples was confirmed through the pathology report in the electronic health records and was defined as insufficiency of acquired tissue for histological analyses. Electronic health records were also used to obtain data

on demographic factors, personal health history, history of obstetric or gynecologic (OB/GYN) surgery (e.g., c-section, tubal ligation, cervix surgery), parity, history of vaginal delivery, and clinical indications for biopsy (e.g., abnormal bleeding, postmenopausal bleeding). Medical records were reviewed for up to a year after the initial Pipelle biopsy attempt following to extract data on the ultimate pathological diagnosis. The biopsy experience forms were also self-administered prior to the biopsy. These forms employed classic, well-validated, and widely used 0-10 numerical rating scales to obtain information on patients' anxiety and pain severity levels, with anchors defined as: 0 = experiencing no anxiety/pain, and 10=experiencing as much anxiety/pain as there could be[154,155].

4.2.2 Variables of interest

Procedure failure, sample inadequacy, and overall Pipelle biopsy failure were each included in statistical analyses as binary (Failure vs. Success) outcome variables, while demographic factors, patient health history, patient experience variables, and clinical indications for biopsy were included as predictor variables. History of vaginal delivery (No vs. Yes) was defined by having at least one vaginal delivery. Women were considered postmenopausal if their medical records indicated that they were postmenopausal, or their age was 52 or above (to ensure postmenopausal status based on ACOG guidelines)[156]. If data on Pipelle procedure failure were missing from the provider questionnaire, corresponding data extracted from the medical records were used. Pain and anxiety levels collected from the questionnaires as continuous variables were further dichotomized as moderate to severe (≥4) and none to mild (<3).

4.2.3 Statistical analysis

Data were analyzed using descriptive statistics, Student's t-test, Mann-Whitney U test, Fisher's exact test, and Chi Square test for continuous and discrete variables as appropriate. Procedure failure (n=22) and overall failure (n=35) were analyzed in total sample (n=121), while sampling inadequacy (n=13) was analyzed in a sample with procedure failures removed (n=99). Factors that were significantly different between failed and successful biopsies were further analyzed using univariable (odds ratio [OR]) logistic regression. The variables that were significant in the univariable regression were included in multivariable logistic regression models (adjusted odds ratio [AOR]) to identify factors significantly associated with procedure failure, sample inadequacy, and overall biopsy failures as separate outcomes. The variables in the in the multivariable models were further selected based on statistical and clinical significance to be included in the final models for each type of Pipelle biopsy failure. Statistically insignificant risk factors from univariable analysis were excluded from the final multivariable regression models. A two-tailed $P \leq 0.05$ was considered significant. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

4.3 Results

Patients' personal and clinical information are presented in Table 6. Participants in the study were predominantly middle-aged (50.87±8.65 years), white (84%; 100/119), and obese (BMI 31.70, IQR 26.22; 36.36). The incidence of procedure failure for Pipelle biopsy was 18% (22/121), while sample inadequacy incidence was 11% (13/121), which yielded a 29% (35/121)

incidence of overall Pipelle biopsy failure. More than half (54.7%; 65/120) of the patients had a history of OB/GYN surgery. The majority of surgeries included C-sections (45%; 29/65), tubal ligations (26%; 17/65), oophorectomies (11%; 7/65), and endometriosis excisions (5%; 3/65). Clinicians reported stenosis as the main reason for Pipelle procedure failure (50%; 11/22). Body habitus (32%; 7/22) and pain intolerance of the procedure (18%; 4/22) were additional reasons reported by clinicians for procedure failure. In comparison, 54% (7/13) of sample inadequacy resulted from scant or fragmented endometrial tissues and 46% (6/13) resulted from sample consisting predominantly blood, fibrin, necrotic tissue, or absence of endometrial tissue. Neither patients' self-reported anxiety nor pain were found to be related to procedure failures, sample inadequacies, or overall Pipelle biopsy failure.

The personal and clinical factors significantly associated with procedure failure, sample inadequacy, and overall Pipelle biopsy failure are presented in Table 6. Age was significantly different for all three Pipelle outcomes. The history of vaginal delivery (P=0.0281), number of previous OB/GYN surgeries (P=0.0100), history of biopsy failure (P=0.0099), menopausal status (P=0.0011), and biopsy indication (P=0.0026) were significantly different between patients who had procedure failure compared to patients who had successful biopsies, whereas overall biopsy failure patients were different from successful biopsy patients based on the history of vaginal

Table 6. Comparison of personal and clinical characteristics of study participants between different types of biopsy failure

	Total (N=121)	Procedure Failure (N=121)			Sample Inadequacy (N=99)			Overall Pipelle Biopsy Failure (N=121)		
		Failure (N=22)	Success (N=99)	P	Failure (N=13)	Success (N=86)	P	Failure (N=35)	Success (N=86)	P
Variable										
Age (SD) (N=121)	50.87 (8.65)	56.68 (10.04)	49.59 (7.80)	0.0004[a]	53.62 (3.78)	48.98 (8.08)	0.0450[a]	55.54 (8.35)	48.98 (8.08)	0.0001[a]
Race (N=119)										
White	100 (84.03%)	20 (90.91%)	80 (82.47%)	0.5208[b]	10 (76.92%)	70 (83.33%)	0.6943[b]	30 (85.71%)	70 (83.33%)	0.7466[d]
Other	19 (15.97%)	2 (9.09%)	17 (17.53%)		3 (23.08%)	14 (16.67%)		5 (14.29%)	14 (16.67%)	
BMI (IQR) (N=119)	31.70 (26.22; 36.36)	33.10 (27.80; 38.04)	31.57 (25.89; 36.13)	0.4572[c]	32.10 (23.49; 37.45)	31.43 (26.33; 35.51)	0.9382[c]	32.49 (24.98; 38.04)	31.43 (26.33; 35.51)	0.5609[c]
History of vaginal delivery (N=119)										
No	41 (34.45%)	12 (54.55%)	29 (29.90%)	0.0281[d]	6 (46.15%)	23 (27.38%)	0.1991[b]	18 (51.43%)	23 (27.38%)	0.0119[d]
Yes	78 (65.55%)	10 (45.45%)	68 (70.10%)		7 (53.85%)	61 (72.62%)		17 (48.57%)	61 (72.62%)	
Diabetes (N=116)										
No	102 (87.93%)	20 (95.24%)	82 (86.32%)	0.4599[b]	11 (84.62%)	71 (86.59%)	1.0000[b]	31 (91.18%)	71 (86.59%)	0.7549[b]
Yes	14 (12.07%)	1 (4.76%)	13 (13.68%)		2 (15.38%)	11 (13.41%)		3 (8.82%)	11 (13.41%)	
History of fibroids (N=119)										
No	56 (47.06%)	9 (40.91%)	47 (48.45%)	0.5221[d]	4 (33.33%)	43 (50.59%)	0.2629[b]	13 (38.24%)	43 (50.59%)	0.2226[d]
Yes	63 (52.94%)	13 (59.09%)	50 (51.55%)		8 (66.67%)	42 (49.41%)		21 (61.76%)	42 (49.41%)	

Table 6 Continued

Ablation history (N=117)										
No	111 (94.87%)	19 (86.36%)	92 (96.84%)	0.0794[b]	12 (92.31%)	80 (97.56%)	0.3602[b]	31 (88.57%)	80 (97.56%)	0.0646[b]
Yes	6 (5.13%)	3 (13.64%)	3 (3.16%)		1 (7.69%)	2 (2.44%)		4 (11.43%)	2 (2.44%)	
History of OB/GYN Surgery (N=120)										
No	55 (45.83%)	5 (22.73%)	50 (51.02%)	0.0161[d]	8 (61.54%)	42 (49.41%)	0.4153[d]	13 (37.14%)	42 (49.41%)	0.2202[d]
Yes	65 (54.17%)	17 (77.27%)	48 (48.98%)		5 (38.46%)	43 (50.59%)		22 (62.86%)	43 (50.59%)	
History of biopsy failure (N=121)										
No	115 (95.04%)	18 (81.82%)	97 (97.98%)	0.0099[b]	13 (100%)	84 (97.67%)	1.0000	31 (88.57%)	84 (97.67%)	0.0575[b]
Yes	6 (4.96%)	4 (18.18%)	2 (2.02%)		0 (0%)	2 (2.33%)		4 (11.43%)	2 (2.33%)	
Menopausal status (N=121)										
Premenopausal	80 (66.12%)	8 (36.36%)	72 (72.73%)	0.0011[d]	8 (61.54%)	64 (74.42%)	0.3335[b]	16 (45.71%)	64 (74.42%)	0.0025[d]
Postmenopausal	41 (33.88%)	14 (63.64%)	27 (27.27%)		5 (38.46%)	22 (25.58%)		19 (54.29%)	22 (25.58%)	
Ultrasound prior to biopsy (N=121)										
No	34 (28.10%)	6 (27.27%)	28 (28.28%)	0.9240[d]	2 (15.38%)	26 (30.23%)	0.3400[b]	8 (22.86%)	26 (30.23%)	0.4131[d]
Yes	87 (71.90%)	16 (72.73%)	71 (81.61%)		11 (84.62%)	60 (69.77%)		27 (77.14%)	60 (69.77%)	
Biopsy indication (N=121)										
Abnormal bleeding	75 (61.98%)	7 (31.82%)	68 (68.69%)	0.0026[d]	8 (61.54%)	60 (69.77%)	0.4840[d]	15 (42.86%)	60 (69.77%)	0.0169[d]

Table 6 Continued

Other	9 (7.44%)	4 (18.18%)	5 (5.05%)		0 (0%)	5 (5.81%)		14 (11.43%)	5 (5.81%)	
Postmenopausal bleeding	37 (30.58%)	11 (50.00%)	26 (26.26%)		5 (38.46%)	21 (24.42%)		16 (45.71%)	21 (24.42%)	
Anxiety prior to biopsy (N=120)										
No to mild	58 (48.33%)	10 (45.45%)	48 (48.98%)	0.7649[d]	4 (30.77%)	44 (51.76%)	0.1584[d]	14 (40.00%)	44 (51.76%)	0.2411[d]
Moderate to severe	62 (51.67%)	12 (54.55%)	50 (51.02%)		9 (69.23%)	41 (48.24%)		21 (60.00%)	41 (48.24%)	
Pain prior to biopsy (N=119)										
No to mild	91 (76.47%)	16 (72.73%)	75 (77.32%)	0.6466[d]	9 (69.23%)	66 (78.57%)	0.4834[b]	25 (71.43%)	66 (78.57%)	0.4026[d]
Moderate to severe	28 (23.53%)	6 (27.27%)	22 (22.68%)		4 (30.77%)	18 (21.43%)		10 (28.57%)	18 (21.43%)	

[a]Student's T test
[b]Fisher's exact test
[c]Kruskal–Wallis test
[d]Chi Square test

delivery (P=0.0119), menopausal status (P=0.0025), and biopsy indication (P=0.0169). There were no significant differences in biopsy failure rates between clinics (data not shown).

The univariable logistic regression analyses are presented in Table 7 and demonstrate that procedure failure was positively associated with older age (OR=1.11, P=0.0013), having no history of vaginal delivery (OR=2.81, P=0.0319), having a history of OB/GYN surgery (OR=3.84, P=0.0139), having a history of biopsy failure (OR=10.78, P=0.0085), being postmenopausal (OR=4.67, P=0.0020), and biopsy indication (other vs. abnormal bleeding: OR=7.77; postmenopausal bleeding vs. abnormal bleeding: OR=4.11, P=0.0065). Sample inadequacy was positively associated with increasing age, with risk increasing by 10% for each additional year of patient's age (OR=1.10, P=0.0498). Overall biopsy failure was associated with patient's age (OR=1.10, P=0.0005), having no history of vaginal delivery (OR=2.81, P=0.0134), being postmenopausal (OR=3.46, P=0.0031), and biopsy indication (other vs. abnormal bleeding OR=3.20; postmenopausal bleeding vs. abnormal bleeding OR=3.05, P=0.0253) in univariable analyses.

The multivariable logistic regression analyses are presented in Table 7. The final model for predicting biopsy procedure failures was fitted to include independent contributions of patient's age (AOR=1.13, P=0.0061), having no history of vaginal delivery (AOR=3.71, P=0.0232), having a history of OB/GYN surgery (AOR=6.21, P=0.0099), and biopsy indication (other vs. abnormal bleeding AOR=14.66; postmenopausal bleeding vs. abnormal bleeding AOR=1.35, P=0.0246). For predicting sample inadequacies, no variables were significant in the final model, other than age. The final model for predicting overall Pipelle biopsy failure included independent contributions of patient's age (AOR=1.12, P=0.0002) and having no history of vaginal delivery (AOR=3.70, P=0.0050).

Table 7. Variables significantly associated with attempted Pipelle biopsy outcomes in univariable and multivariable analyses

	Univariable logistic analyses		Multivariable logistic analyses	
Variable	OR (95% CI)	P	AOR (95% CI)	P
Outcome: procedure failure (ref. success)				
Age	1.10 (1.04, 1.17)	0.0013	1.13 (1.04, 1.23)	0.0061
History of vaginal delivery (ref. yes)	2.81 (1.09, 7.24)	0.0319	3.71 (1.20, 11.51)	0.0232
History of OB/GYN Surgery (ref. no)	3.54 (1.21, 10.35)	0.0209	6.21 (1.55, 24.88)	0.0099
History of biopsy failure (ref. no)	10.78 (1.84, 63.29)	0.0085	NS*	-
Menopausal status (ref. premenopausal)	4.67 (1.76, 12.37)	0.0020	NS	-
Biopsy indication (ref. abnormal bleeding)		0.0065		0.0246
Other	7.77 (1.69, 35.81)		14.66 (2.08, 103.34)	
Postmenopausal bleeding	4.11 (1.44, 11.74)		1.13 (0.25, 5.03)	
Outcome: sample inadequacy (ref. success)				
Age	1.10 (1.00, 1.17)	0.0498	1.10 (1.00, 1.17)	0.0498
Outcome: overall Pipelle biopsy failure (ref. success)				
Age	1.10 (1.04, 1.16)	0.0005	1.12 (1.05, 1.18)	0.0002
History of vaginal delivery (ref. yes)	2.81 (1.24, 6.36)	0.0134	3.70 (1.49, 9.21)	0.0050
Menopausal status (ref. premenopausal)	3.46 (1.52, 7.87)	0.0031	NS	-
Biopsy indication (ref. abnormal bleeding)		0.0253	NS	-
Other	3.20 (0.77, 13.39)			
Postmenopausal bleeding	3.05 (1.29, 7.22)			

*NS – not significant in the final model

4.4 Discussion

To our knowledge, this is the first prospective study to separately examine both procedure failure and sample inadequacy factors associated with failed Pipelle biopsies with data acquired from both clinician feedback and pathology records in a routine clinical setting. We found incidences of 18% for procedure failure and 11% for sample inadequacy, resulting in a total of 29% incidence for overall biopsy failure. Regression analyses demonstrated that these different attempted Pipelle biopsy outcomes had different predictors. In particular, older age, history of vaginal delivery, history of OB/GYN surgery, and biopsy indication were significantly related to procedure failure, while only age was an important predictor for sample inadequacy. Overall

biopsy failure was associated with age and history of vaginal delivery. We suggest that the results of this study could be used by practicing clinicians and hospital managers to reduce the patient and hospital burden resulting from failed diagnostics by pursuing alternative approaches (e.g., D&C) in patients at high risk for Pipelle biopsy failure.

Procedure failure and sample inadequacy rates substantially varied across previous Pipelle biopsy studies published over the last two decades, in part reflecting differences in the definitions of failure used. In the present study, we found 29% incidence for overall biopsy failure, which is a little higher than we found in our previous medical records review study, where we reported 23% overall failure rate in a general population sample[77]. In comparison, earlier studies reported biopsy failure rates of 10% for inability to acquire sample (procedure failure) and another 10% for inadequacy of acquired sample[150], while a 2002 systematic review by Clark et al. reported a range of 0-22% procedure failure rates and a range of 0-76% sample inadequacy rates[129]. In a more recent study, Visser et al. reported 20.8% technical (procedure) failure and 29.8% tissue insufficiency (sample inadequacy) in women with postmenopausal bleeding[157], whereas Xie reported 2.1% procedure failure rate and 5.9% sample inadequacy[153]. In another study, Piatek et al. reported 17.3% insufficient (inadequate) sample but did not evaluate procedure failure[158].

We expected higher overall biopsy failure rates for the present study because our previous study did not capture procedure failures that were not recorded in patients' medical records[77], which was possible for this prospective study. Considering the results of the present study and previously published studies, we suggest that failure rates depend on the patient sample selection, definitions of success, and clinical settings. For example, the Visser et al. study analyzed failure rates in women with postmenopausal bleeding[157], which would explain higher rates compared to our study, which included both pre- and post-menopausal women. On the other hand, in a

prospective study by Xie et al., Pipelle biopsy was followed-up immediately by D&C[153], which suggests use of anesthesia for both procedures and, therefore, lower failure rates compared to the current study due to increased tolerance of the procedure.

In the present study, we demonstrate that older age, history of vaginal delivery, history of OB/GYN surgery, and biopsy indication were significant predictors of procedure failure, whereas age was predictive of both sampling inadequacy and overall biopsy failures. These findings are similar to our previous study, where we identified biopsy indication, history of prior biopsy failure, and provider type to be associated with overall biopsy failure as indicated by medical record review[77]. Biopsy indication remained important in the current study, while prior history of biopsy failure was only significant in univariable analysis.

Our findings in the present study were also in line with the study by Visser et al., which reported significant association of age with technical (procedure) failure (AOR=1.03) and insufficient (inadequate) sample (AOR=1.04)[157], whereas in our study, we report AORs of 1.11 and 1.10 for procedure failure and sample inadequacy, respectively. Stronger effect of age in our cohort can be potentially explained by differences in the patient samples. Visser et al. included only postmenopausal women in their study sample[157], whereas the present study included both pre- and post-menopausal women.

The literature is inconsistent regarding association between parity and Pipelle biopsy failure, with some studies reporting higher procedure failure rate in nulliparous women[157,159], while other studies report no such association[160]. This inconsistency may be the result of using parity instead of history of vaginal delivery, which changes the anatomy of the uterus and allows easier access to the uterine cavity[161]. We have found that having no history of vaginal delivery significantly increases the risk of procedure failure, which is concordant with what was reported

by Xie et al., although the heightened odds ratio in that study was not statistically significant[153]. We have also found that history of OB/GYN surgery is significantly associated with procedure failure, which is consistent with previous findings of Xie et al., who reported negative effect of cervical surgeries and positive effect of intrauterine procedures[153].

Our results suggest that almost one third of the Pipelle biopsies failed due to procedure failure or sample inadequacy, which exceeds what has been traditionally considered the expected rate of failure[150]. It might be important for physicians and hospital managers to closely monitor Pipelle failures, as these procedures are common and failures might contribute to a large burden of unrecognized disease[138]. Moreover, our results indicate that procedure failures and sample inadequacy are associated with different risk factors, suggesting that a variety of strategies are needed for patients with different medical histories to reduce the risk of overall Pipelle biopsy failure. For example, it may be recommended to refer elderly patients with prior history of OB/GYN surgery and no history of vaginal delivery directly to D&C to avoid additional Pipelle failure and save time and resources for both patient and clinical providers.

This study fills an important gap in our understanding of predictors of Pipelle biopsy failure and reports clinically relevant incidences of procedure failure and sample inadequacy. However, it is important to highlight the lack of consistency in definitions of biopsy failure across the research literature[129], inconsistencies in what is considered a histopathologic failure [123,137], and lack of recent meta-analyses. Future studies should contribute to the literature by concentrating on improving the current protocols for Pipelle biopsy procedures to adjust for the factors that increase the risk of biopsy failure.

There are several strengths in our present study. To our knowledge, this is the first study to collect data independently from provider and medical records and use this information to

identify rates and factors associated with procedure failure and sample inadequacy, as well as overall Pipelle biopsy failure. In addition, the data are prospective, as we identified outcomes of the Pipelle biopsy by following up on recruited patients, which improves our understanding of care trajectory for patients undergoing Pipelle biopsy, while existing studies on Pipelle biopsies have been predominantly retrospective, drawing data from patient medical records alone[77,126,128,134,149,162]. Another strength is that the data were collected from both primary care and specialty clinics, allowing us to include a variety of patients and indications for biopsy. An additional strength of the study was our collection of provider questionnaire data immediately after the procedure, which reduces the chance of recall bias or confounding by knowledge of pathology results. Also, our data collection was naturally integrated into the normal clinical workflow, which potentially improved clinical applicability of the results and reduced the biases associated with having a sample recruited outside of the clinical context.

The major limitation of this study is the lack of ability to analyze the role of ultrasound endometrial thickness in biopsy failure[140,153,157], as an ultrasound was done in only 72% of patients that underwent Pipelle biopsy. Moreover, it should be noted that the failure rates here may be lower than those seen in other ordinary clinical settings since all biopsies were conducted by highly trained OB/GYN physicians. However, there were no significant differences in failure rates between clinicians from specialized clinics compared to primary care clinics in the present study (data not shown). Additionally, there is always some potential for residual confounding from factors that were not assessed.

4.5 Conclusions

In summary, we have reported the incidence of Pipelle procedure failure, sample inadequacy, and overall biopsy failure in a routine clinical practice setting, as well as explored factors associated with these different clinical outcomes. Reducing the number of Pipelle failures should be a priority for both healthcare clinicians and managers, since a one in five chance of uterine pathology has been reported in a study of biopsies conducted following an initial failed biopsy[138]. If present findings are confirmed in subsequent research in other diverse clinical settings, we suggest adjustments to the existing Pipelle biopsy guidelines to encourage clinicians to consider predictors of biopsy failure when deciding which diagnostic route to pursue.

Significance. The results of this study might potentially inform physicians and create updated guidelines on the appropriateness of procedure for a particular patient, given the medical history, as well as pain and anxiety levels experienced by the patient.

Novelty. This is the first study to analyze the effects of both personal and clinical factors on the success rate of Pipelle biopsy.

Aim. The aim of this study is to acquire a better estimation of endometrial Pipelle biopsy failure in an outpatient setting, as well as to identify additional patient factors that might affect the success rate of Pipelle biopsy.

Paper status. The manuscript is currently being prepared for resubmission

5.0 Synthesis

This research work addresses major gaps in the current literature pertaining to EC diagnostics. We argue the importance of holistic approach to EC diagnostics, i.e. including both, physical and mental aspects of approaching cancer diagnostics in particular patient. To this end we developed Holistic Model for Diagnostics of Endometrial Cancer (HOMDEC) framework which is aimed to serve as decision support tool for improving identification and diagnostics of EC based on the results of this research work (Figure 2).

The provider which is managing the patient with suspected endometrial pathology can use HOMDEC framework to identify the potential risk of EC and sampling failure. This framework describes the stages starting from (1) endometrial biopsy indications; (2) going through the decision tree based on patient MetS and obesity status; (3) assessing the risk factors for Pipelle failure; (4) deciding optimal diagnostic path; and (5) arriving at diagnosis, while placing women in high or low EC risk groups based on the patient characteristics and risk of Pipelle failure. The initial stages (1-2) of HOMDEC framework are well supported by the existing literature on the association of EC with menopause and MetS[48,122]. Later stages (3-5) are supported through published findings of this dissertation work[77], as well as a number of other articles on the topic of Pipelle biopsy failure[125,153,157,159].

The HOMDEC framework does not significantly expand the time for patient management, as the first stages (1-3) can be easily automated by incorporating into medical records, as information is already there. For example, a special algorithm can identify if patient has MetS based on the results of physical measurements, prescription drug history, and routine blood biochemistry tests. The main hurdle is general lack of waist circumference measurement in

electronic medical records, which is one of the components of Harmonized and IDF MetS definitions[163]. This can be addressed by temporary using BMI and incorporating waist circumference measurement into routine clinical care further down the line, as waist circumference is a better measurement of excess adiposity compared to BMI[105].

The later stages (4 and 5) of HOMDEC framework require more careful consideration as provider would have to take into account the history of prior biopsy failure, levels of obesity, and patient experiences during the prior biopsy. On the other hand, these considerations are more crucial for later stages, as they will allow a systematic approach to endometrial sampling, which would produce the best outcomes for the patient, as well as reduce the discrepancies in the follow-up trajectories potentially saving patient management time. Overall, the HOMDEC framework will not add significantly to the patient management time even adjusting for potential additional considerations for the provider, because the decision making is straightforward and binary once the flow and logic of the model are understood.

The first step of the first stage identifies women experiencing symptoms suggestive of endometrial pathology, such as abnormal uterine bleeding, which is responsible for more than two-thirds of all peri- and post-menopausal gynecological visits[164]. The second step of the first stage is to assess menopausal status as women after menopausal transition are at higher risk of EC[165] and Pipelle biopsy failure[77].

The second stage of HOMDEC framework focuses on assessing the MetS and obesity status of the patient. Currently MetS is not used for clinical diagnosis of EC or risk evaluation. This might have resulted from the lack of universally accepted MetS definition and discrepancies in scientific evidence resulting from differences in MetS definition used in research. Paper 1

demonstrated that different definitions of MetS lead to differences in the prevalence of MetS in EC patients[163].

Identifying MetS status as a way of assessing potential risk of EC for a particular patient might prove clinically useful, given availability of appropriate prospective research and single unified definition that can be used to link results from research studies and clinical practice. We found that IDF and Harmonized definitions are more useful for EC compared to other definitions. While these definitions are not particularly developed for predicting risk of EC, published evidence demonstrated that they have sufficient association with EC to warrant their use in diagnostics of EC[48]. Therefore, the results of this research work suggest that there is no need to develop MetS definition specific to EC. However, there is definite need to use a single MetS definition for EC research and clinical purposes.

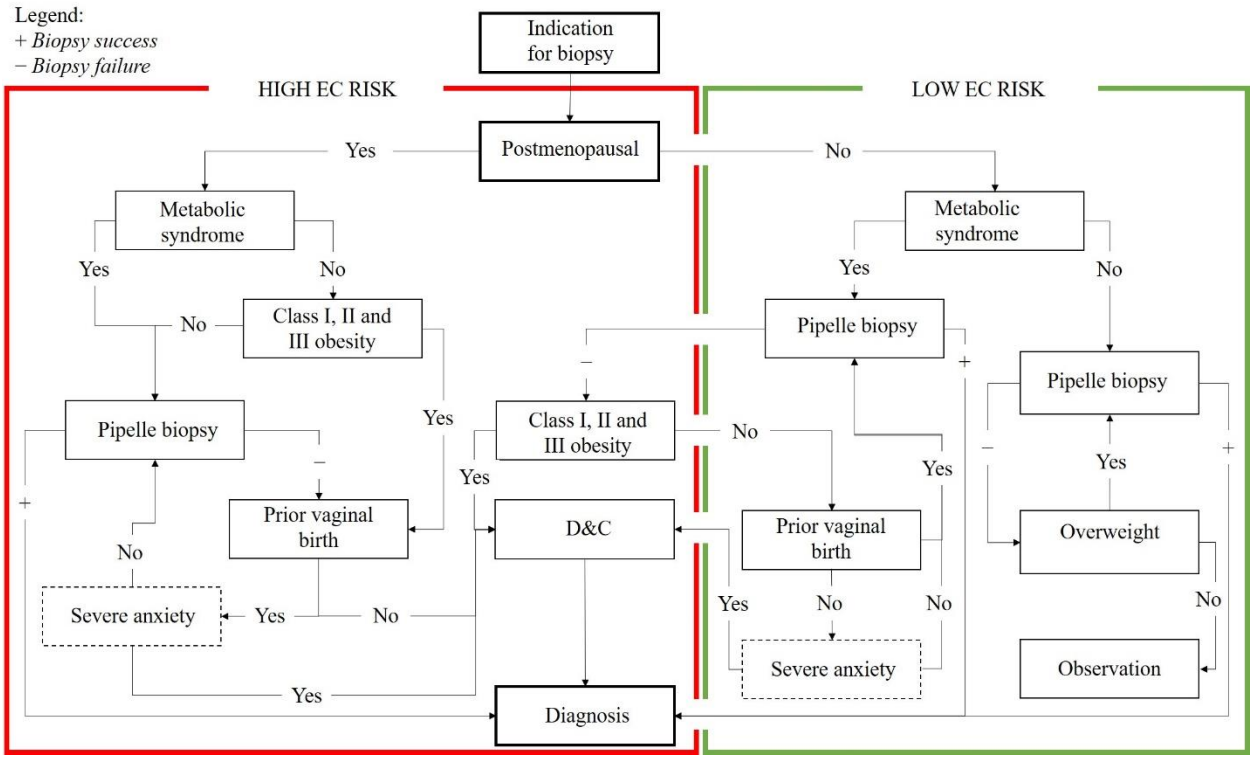

Figure 2. Holistic Model for Diagnostics of Endometrial Cancer (HOMDEC)

Diagnosis of MetS does not always require abdominal obesity, and therefore assessing obesity status of the patient becomes important for diagnostic model, since it the main risk factor for EC among other MetS components[48]. There is a strong dose–response relationship between obesity and EC, as OR of developing EC for overweight (BMI 25-29.9) was 1.5, for class I obesity (BMI 30–34.9) was 2.5, for class II obesity (BMI 35-39.9) was 4.5 OR, and for a class III obesity (BMI ≥ 40) was 7.1 when compared to normal weight populations (BMI<25)[8]. Moreover, Calle et al. demonstrated that relative risk of death for overweight (BMI 25-29.9) women with EC was 1.50, for class I obesity (BMI 30–34.9) was 2.53, for class II obesity (BMI 35-39.9) was 2.77, and for a class III obesity (BMI ≥ 40) was 6.25 compared to normal weight women[166]. Therefore, women with class I, II, and III obesity are considered high EC risk in HOMDEC framework.

Steps three and four include assessment of Pipelle biopsy risk factors and deciding which diagnostic route (Pipelle/D&C) to choose. Papers 2 and 3 presented in this research work made major contributions to this part of the decision tree. Pipelle biopsy was chosen as the primary diagnostic procedure in HOMDEC framework because it is already the widely used endometrial pathology diagnostic modality and more cost-effective than D&C, even after adjustment for biopsy failure[146]. However, in the cases of repeat Pipelle failures or considerable risk of Pipelle biopsy failure, patients can be advised to undergo D&C directly to avoid additional costs and patient burden. Although this dissertation work does not include data on follow-up of women with failed biopsy, our group is currently working on identifying trajectories of care among women with failed biopsies. Specifically, among women with overall biopsy failures, follow-up procedures and/or counseling have already been conducted in the majority of patients and found to include: hysteroscopy/D&C (33%), another Pipelle (4%), Pipelle and D&C (4%), hysterectomy without additional endometrial sampling (19%), ultrasound (4%), and no procedure (37%). Among women

with follow-up, approximately one third had pathological findings, which included endometrial polyps (29%) and cancer (6%).

The final stage is to provide patient with a diagnosis, which is especially important for women in the high EC risk group, or continue with observation if the patient is in the low EC risk group (in this case, the burden of diagnostic procedures will be higher compared to low risk of endometrial pathology).

Overall, the three papers outlined in this research as well as unpublished data from prospective Pipelle project significantly contribute to the HOMDEC framework. This framework suggests a novel decision tree for stages involved in diagnosing EC and other endometrial pathologies, while using previously published scientific evidence on risk factors associated with EC and Pipelle biopsy failure factors. HOMDEC framework can be used as decision support tool for providers who perform endometrial sampling using Pipelle or D&C, aiming at maximizing the diagnostic potential of endometrial biopsy as well as reducing its cost and burden to both patient and healthcare system. Quick and reliable diagnostics proposed by HOMDEC framework can be especially useful in overweight and obese patients, taking into account these patients have a higher chance to delay and avoid medical care[106].

6.0 Novelty and Public Health Significance

EC is the 4th most common cancer in US women and is the most common gynecologic malignancy among women in the developed world[167]. The incidence of EC has risen by approximately 25% over the past decade[1] and is expected to continue the upward trend[5], a phenomenon which has primarily resulted from the epidemic of obesity. Moreover, there is a strong evidence linking EC with obesity and diabetes, suggesting that EC is sensitive to abnormal metabolism. A previously published systematic review established MetS as a risk factor for EC, although there is a considerable discrepancy between MetS definitions[48]. Therefore, identifying women at higher risk of EC becomes increasingly important, which can be assisted by using the singular definition of MetS. While screening for EC is not recommended in the general population[92,168], we suggested that it may need to be implemented in high risk populations, such as women with obesity and/or MetS. Though obesity is the main risk factor for EC, other components of MetS can also play significant roles in development of EC[122]. In particular, meta-analysis of the association between each MetS component and EC demonstrated that the majority of components are significant risk factors for EC, including BMI/waist circumference (RR=2.21), hyperglycemia (RR=1.81), blood pressure (RR=1.81), and triglyceride level (RR=1.17), whereas HDL-cholesterol was not significant[48]. However, despite abundant literature suggesting the association between MetS and EC, MetS is not routinely used as a risk factor for clinical detection and diagnosis of EC[48]. This research work recognizes the importance of MetS in EC/endometrial pathology detection pathway and includes it in the novel HOMDEC framework, as a new way of looking at diagnostic path for EC.

The other important issue is that one of the most commonly used diagnostic procedures, Pipelle endometrial biopsy, has a failure rate of 38% in women with obesity[16], compared to 23% failure rate in more general population[77]. This is a serious issue, as overweight and patients with obesity were shown to delay and avoid medical care[106]. Arriving at a diagnosis as quickly as possible with fewer diagnostic procedure failures becomes critically important for this type of patients, as they are less likely to keep future appointments, leading to a reduced number of options for treatment. As a result, there is a pressing need to identify women at high risk of EC and improve success rate of diagnostic procedures in women with obesity. This research work identified both patient and provider factors associated with Pipelle biopsy failure, which can help to improve the success rate of this procedure, and consequently provide much needed improvements in the value of care for EC diagnostics. Therefore, HOMDEC pays special attention to Pipelle biopsy failure, as timely and successful identification of EC is critical, especially in high EC risk populations.

This research is one the first to explore patient, provider, and health system factors that may influence the rate of Pipelle biopsy use and failure within a single study. Improving knowledge about these factors can have significant policy implications by providing a foundation for programs of screening and preventive strategies for EC. Moreover, this research work and HOMDEC framework can potentially reduce barriers to the effective implementation of Pipelle biopsy in medical office settings, improving the "value" of care (improving patients' outcomes while reducing costs), especially for women with obesity and MetS.

The long-term goal of our research is to identify improved ways to detect EC and other endometrial pathologies timely and efficiently. Future studies should focus on prospectively collecting data from larger samples of patients and healthcare systems to identify the failure rate across the general population and collect more information that may predict which patients are

most likely to have a successful biopsy. Additionally, the potential of HOMDEC to be adapted into routine gynecologic oncology practice should be investigated to improve the value of care and improve minimally invasive EC screening. In summary, our future research will lead to improving value of care for women with suspected endometrial pathologies by reducing the number of invasive and risky D&C operations (and side effects associated with those), while controlling the cost of care.

This research work was aimed to explore the holistic approach to diagnosing and preventing EC by exploring multiple data sources including patient, provider, and health system factors, as well as to develop comprehensive diagnostic model for EC based on the results of this research work. Moreover, this work addressed the clinically important issue of Pipelle biopsy failure and, to our knowledge, is the first research effort to prospectively explore factors associated with biopsy failure in routine clinical practice. There is a pressing need to improve success rate of endometrial diagnostic procedures in high risk women. Understanding factors associated with Pipelle biopsy failure can facilitate personalized approach to this procedure and improve success rate. These research results meld current knowledge of epidemiologic risk factors and endometrial diagnostic procedures implicated in EC and other endometrial pathologies. Additionally, the results of this research work can be adapted by developing countries, such as Kazakhstan, where Pipelle use is not widespread, but EC pose a significant public health challenge. By introducing Pipelle and HOMDEC in these countries we can potentially reduce EC mortality and improve efficiency of EC diagnostic procedures.

The future research should concentrate on applying the findings of this research work to clinical practice after consulting with physicians, nurses, and hospital administration. In particular, the HOMDEC framework might be useful for providers practicing Pipelle and/or D&C. As a

result, the next steps after of completion of this research work would be analyzing and publishing data on patient experiences during and after the Pipelle biopsy. Consequently, all of the research work from our group and previously published research will be incorporated into updated HOMDEC model, which will be further discussed with providers to improve its applicability and adaptability. The overall results of this research work would help me to build necessary expertise to conduct applied epidemiology research aimed at improving diagnostics, patient treatment, and effectiveness of healthcare.

Appendix A Provider survey

Post biopsy provider survey. This survey will be completed on the day of the Pipelle procedure.

1. For the patient today, what was the indication for the biopsy? (check all that apply)

- ❑Postmenopausal bleeding
- ❑Irregular bleeding
- ❑Cystic lining of the uterus
- ❑Menorrhagia
- ❑Abnormal cells on pap smear
- ❑Tamoxifen use with bleeding problems
- ❑Follow up to a prior abnormal biopsy
- ❑Thickened endometrial lining on the ultrasound
- ❑Other (specify) _____

2. Did this patient have uterine ultrasound prior to today's visit?

- ❑Yes
- ❑No

3. What was the previous Pipelle sampling history for this patient? (check one)

- ❑This was the first attempt to sample the endometrium for this patient.
- ❑Previous endometrial tissue collection was successful
- ❑Previous endometrial tissue collection was not successful
- ❑Previous endometrial tissue collection history is unknown
- ❑Other (specify) _____

4. For this patient, what endometrial sampling procedure did you recommend?

- ❍Pipelle
- ❍D&C

5. What procedure did the patient agree to?

❍Pipelle (if yes is checked, go to questions 6)
❍D&C (if no is checked, answer questions 10 and 11 and then survey is completed. Thank you!)

6. For the Pipelle attempted today, did you use any anesthetics?

❍Yes (indicate anesthetic type) _____
❍No

7. For the Pipelle attempted today, were you successful in obtaining a sample?

❍Yes (if yes is checked, survey is completed. Thank you!)
❍No (if no is checked, answer questions 8 and 9 and then survey is completed. Thank you!)

8. If Pipelle biopsy was attempted today and a sample was not collected, please indicate why this attempt was not successful. (Check all that apply)

❑BMI (severe obesity)/ Body habitus making the Pipelle procedure difficult
❑Disability (impossible for the patient to climb the chair)
❑Pain intolerance
❑Anxiety level of patient
❑Abnormal uterine structure
❑Developmental anomaly
❑Stenosis
❑Inability to locate cervix
❑Speculum not long enough
❑Other (specify)_____

9. If Pipelle was not successful, will you reschedule Pipelle for a different day?

❍Yes, with cervical ripening agent
❍Yes, no cervical ripening agent
❍Yes, other management strategy (specify)_____
❍No, patient will have D&C
❍No, other management strategy (specify)_____

10. For the patient today, if D&C sampling in OR was chosen without attempting Pipelle, what were the patient characteristics that made it important to refer her for D&C?

❑History of previous unsuccessful Pipelle biopsies
❑Patient declined Pipelle route
❑Additional uterine problems that can be addressed in the OR setting (such as fibroids, polyps)
❑BMI (severe obesity)/ Body habitus making the Pipelle procedure difficult
❑Disability (impossible for the patient to climb the chair)
❑Pain intolerance
❑Anxiety level of patient
❑Abnormal uterine structure
❑Developmental anomaly
❑Known history of cervical stenosis
❑Other _____

11. For the patient today, if D&C sampling in OR was chosen without attempting Pipelle, what were the office characteristics that contributed to your decision? (check all that apply)

❑No access to large chair
❑No access to long speculum
❑No access to uterine sound or other equipment
❑No access to medical assistant
❑No office characteristics were involved in the decision
❑Other _____

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Appendix B Patient Experience Surveys

Appendix B.1 Biopsy Experience—Before the Biopsy

Below is a list of words that describe feelings people have. Please read each one carefully. Then CIRCLE ONE number that best describes **how you have felt today.**

		Not at all	A little	Moderately	Quite a bit	Extremely
1.	Tense	0	1	2	3	4
2.	On edge	0	1	2	3	4
3.	Uneasy	0	1	2	3	4
4.	Restless	0	1	2	3	4
5.	Nervous	0	1	2	3	4
6.	Anxious	0	1	2	3	4

Now, please think about how you have felt **since you got to the clinic** today.
On a scale of 0 to 10, circle the number that best describes how you felt. **0** means that the symptom was **absent** and **10** means that it was **as strong as possible**.

At its best since getting to the clinic, how relaxed did you feel?

Not at all 0 1 2 3 4 5 6 7 8 9 10 As relaxed as I could be

At its worst since getting to the clinic, how worried were you about the upcoming procedure itself and what will happen during the procedure?

Not at all 0 1 2 3 4 5 6 7 8 9 10 As worried as I could be

At its worst since getting to the clinic, how worried were you about what the procedure results will show?

Not at all 0 1 2 3 4 5 6 7 8 9 10 As worried as I could be

At its worst since getting to the clinic, how anxious did you feel?

Not at all 0 1 2 3 4 5 6 7 8 9 10 As anxious as

												I could be
At its worst since getting to the clinic, how intense was your pain?												
Not at all	0	1	2	3	4	5	6	7	8	9	10	As intense as it could be

Appendix B.2 Biopsy Experience—Thinking Back to During the Biopsy

Please think about how you felt **during the procedure.**
On a scale of 0 to 10, circle the number that best describes how you felt. **0** means that the symptom was **absent** and **10** means that it was **as strong as possible**.

At its worst during the procedure, how anxious did you feel?

Not at all	0	1	2	3	4	5	6	7	8	9	10	As anxious as I could be

At its worst during the procedure, how much discomfort did you feel?

None	0	1	2	3	4	5	6	7	8	9	10	As much discomfort as there could be

At its worst during the procedure, how intense was your pain?

Not at all	0	1	2	3	4	5	6	7	8	9	10	As intense as it could be

At its worst during the procedure, how unpleasant was your pain?

Not at all	0	1	2	3	4	5	6	7	8	9	10	As unpleasant as it could be

At its best during the procedure, how relaxed did you feel?

Not at all	0	1	2	3	4	5	6	7	8	9	10	As relaxed as I could be

Appendix B.3 Biopsy Experience—Before Discharge Home

Please think about how you have felt **since the end of the procedure.**
On a scale of 0 to 10, circle the number that best describes how you felt. **0** means that the symptom was **absent** and **10** means that it is w**as as strong as possible**.

At its worst since the end of the procedure, how anxious did you feel?

Not at all	0	1	2	3	4	5	6	7	8	9	10	As anxious as I could be

At its worst since the end of the procedure, how much discomfort did you feel?

None	0	1	2	3	4	5	6	7	8	9	10	As much discomfort as there could be

At its worst since the end of the procedure, how intense was your pain?

Not at all	0	1	2	3	4	5	6	7	8	9	10	As intense as it could be

At its worst since the end of the procedure, how unpleasant was your pain?

Not at all	0	1	2	3	4	5	6	7	8	9	10	As unpleasant as it could be

At its best since the end of the procedure, how relaxed did you feel?

Not at all	0	1	2	3	4	5	6	7	8	9	10	As relaxed as I could be

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