Investigation and management of women presenting with postmenopausal vaginal bleeding

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Dedicated to my children Pyrros and Ariadne, my partner Alice and my parents Pyrros and Nafsika.

Abstract

Postmenopausal vaginal bleeding (PMB) is a common gynaecological symptom that requires investigation to exclude an underlying malignant cause. Endometrial cancer is the most common malignancy diagnosed in women investigated for PMB. The risk of a patient being diagnosed with endometrial cancer varies depending on the presence or absence of certain clinical and demographic characteristics. Although all women presenting with PMB are referred urgently for investigation, the majority of the patients will be diagnosed with a benign pathology.

The main aim of the work included in this thesis is to present the development of diagnostic models that predict the risk of endometrial cancer in patients presenting with PMB. In addition, I attempted to quantify the risk of endometrial cancer in particular subgroups of women undergoing investigation for PMB.

I developed the idea for this project during my clinical training in Obstetrics and Gynaecology. Following literature review, I identified the need for new predictive models in patients presenting with PMB. The work presented in this thesis is based on prospective data collection of consecutive patients referred to a hospital clinic for investigation of PMB. In collaboration with my colleagues, I developed and internally validated two diagnostic predictive models, one based on clinical characteristics only and a second model incorporating clinical characteristics and the results of endometrial thickness measurement using transvaginal ultrasonography. Implementation of the predictive models in clinical practice will allow stratification of patients into risk groups and, subsequently, better prioritisation of the diagnostic tests. However, several other stages in the model development process such external validation and impact analysis are required prior to implementation of the models in clinical practice.

In other manuscripts included in this thesis, I evaluated the causes of PMB and quantified the risk of endometrial cancer for young postmenopausal women, women using hormone therapy and those with inadequate assessment of the endometrium on ultrasonography. The results of these studies help to improve our understanding of different steps of the investigation pathways for women with PMB.

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Chapter 1. Introduction

1.1 Endometrial cancer

Incidence

Endometrial cancer is the most common gynaecological malignancy in the United Kingdom (UK), with more than 7900 new cases diagnosed in 2009 [1]. The lifetime risk of developing endometrial cancer in the UK is 2.7% [1]. The age-standardised incidence rate of endometrial cancer has increased by 29% between 1999 and 2009 (21.1 versus 27.3 per 100,000 females, respectively) [1]. The increasing prevalence of obesity is likely to be the most important cause of the observed change in the incidence [2], although other factors may play an important role. For example, the incidence of endometrial cancer is calculated as a proportion of the total female population, which includes women that have undergone hysterectomy within the denominator. As the percentage of women that have undergone hysterectomy for benign gynaecological pathologies has decreased in recent years [3], the true difference in the incidence of endometrial cancer over this time period should be lower than initially estimated.

Histopathology

Bokhman described two types of endometrial cancer that display different pathogenesis, histological characteristics and clinical behavior [4]. Type 1 tumours comprise up to 80% of cases, with endometrioid adenocarcinoma representing the most common histological variant [5]. These tumours are a consequence of excessive oestrogenic stimulation of the endometrium without counteraction by progesterone; this initially leads to development of endometrial hyperplasia [6]. Atypical endometrial hyperplasia often leads to the development of type 1 tumours. Approximately 80% of patients diagnosed with type 1 endometrial cancer present with early-stage disease [7-10]. The overall survival is significantly better for patients with type 1 compared to those with type 2 tumours [11-14].

The most common histological subtypes included in type 2 tumours of the endometrium include serous and clear cell carcinomas. Approximately 10% of patients diagnosed with endometrial cancer will have serous histology [11, 14], while clear cell carcinomas represent 3-5 % of cases [15, 16]. While type 1 tumours are associated with endometrial hyperplasia, type 2 tumours develop on a background of atrophic endometrium [17, 18]. Ambros et al found that atrophic endometrium was more frequently observed in patients diagnosed with serous compared to those with endometrioid carcinoma (76% versus 29% respectively, $p < 0.0001$) [17]. Several precancerous lesions for type 2 tumours have been described [17, 19-22]. Endometrial Glandular Dysplasia (EmGD) and Endometrial Intraepithelial Carcinoma clear cell type have been identified as the most likely true precursor conditions of serous and clear cell carcinoma of the endometrium, respectively [20, 21, 23, 24]. At the time of diagnosis, the majority of patients diagnosed with type 2 tumours will have metastatic disease [25-28].

Risk factors

Certain conditions can increase the risk of developing type 1 endometrial cancer. Obesity and anovulatory conditions such as polycystic ovarian syndrome are associated with an increased risk of endometrial cancer [29, 30]. Excess weight leads to hyperinsulinaemia, decreased levels of sex-hormone binding globulin (SHBG) and to an increase in the peripheral aromatisation of androgens [31-33]. The end result of the above changes is an increase in the levels of the bioavailable oestrogens [30]. Polycystic ovarian syndrome is a condition of hyperinsulinaemia and anovulation, with subsequent decreased ovarian production of progesterones resulting in an increased risk of endometrial cancer [34]. Through similar mechanisms, patients diagnosed with diabetes are at increased risk of developing endometrial cancer [35-37]. Obesity appears to play an important role in the development of type 2 endometrial cancer too. In a population-based study of 1 million Norwegian women the

authors found obesity was associated with an increased risk of type 2 endometrial cancer [38]. The authors did not propose a mechanism to explain the interaction observed, but it is likely that type 1 and 2 tumours have certain common clinical, pathophysiological and molecular characteristics [39].

The majority of cases of endometrial cancer are diagnosed in women older than 50 years [40, 41] and the average age of diagnosis is 63 years [42]. Increasing age is a risk factor for women with type 2 tumours as well and these patients are usually older than patients diagnosed with type 1 endometrial cancer [10]. Other studies however, suggest that the age at the time of diagnosis is not significantly different for patients with type 1 and 2 tumours [38].

Several publications support an association between nulliparity and increased risk of endometrial cancer [43, 44]. However, it remains unclear if nulliparity represents an independent risk factor or if the interaction found was as a result of infertility due to annovulatory cycles [45]. A history of hypertension has also been found to be a risk factor for developing endometrial cancer, although the mechanism of this association is not well understood [46, 47].

Lynch syndrome or hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant condition that is associated with an increased risk of developing colorectal, endometrial and ovarian cancer in affected individuals [48, 49]. It is caused by germline mutations in MLH1, MSH2, MSH6 and PMS2 mismatch repair genes [50]. These mutations are detected in 1.8 -2.1% of patients diagnosed with endometrial cancer [51-53], rising to 4.9% to 9% of patients who are diagnosed with endometrial cancer at an age younger than 50 years [53-55]. The lifetime risk of developing endometrial cancer varies depending on the specific mutation [56-59]. In a study of 537 families with germline mutations for Lynch syndrome the cumulative risk of endometrial cancer by the age of 70 years for MLH1, MSH2 and MSH6 gene carriers was 54%, 21% and 16%, respectively [49].

The risk of endometrial cancer is increased in patients with a personal history of breast cancer [60]. Although breast cancer and type 1 endometrial tumours have some common risk factors, a personal history of breast cancer is also associated with an increased risk of developing serous carcinoma of the endometrium [61-64]. In a study of women diagnosed with endometrial cancer, the incidence of serous carcinoma was significantly higher in patients with a personal history of breast cancer compared to those without a history of breast cancer (9.4% versus 6.3%, respectively; $p < 0.001$) [63].

Tamoxifen is a selective estrogen receptor modulator used to treat women diagnosed with breast cancer [65, 66]. It has an antiestrogenic effect on breast tissue, but has an agonistic action on oestrogenic receptors in the postmenopausal uterus [65, 67]. The available literature suggests the risk of endometrial cancer is 2-3 times greater in women using tamoxifen compared to non-users [67, 68].

Young age at menarche and late menopause have been found to be associated with increased risk of endometrial cancer, likely due to increased duration of exposure of the endometrium to excess oestrogens and/or decreased production of progesterone [69-71].

Clinical presentation

More than 75% of patients diagnosed with endometrial cancer present with postmenopausal vaginal bleeding (PMB) [72-76]. In premenopausal women, endometrial cancer symptoms include: persistent change in bleeding pattern, heavy menses, irregularities in the frequency of menses, or intermenstrual bleeding. However, a significant percentage of women with endometrial cancer may be asymptomatic, with disease never detected during their lifetime [77]. Based on assumptions from previous literature it has been estimated that 15% (range 5-20%) of women with endometrial cancer will be asymptomatic [78].

1.2 Postmenopausal vaginal bleeding

Incidence

Postmenopausal vaginal bleeding (PMB) describes any bleeding from the female genital tract after the menopause. In clinical practice, any vaginal bleeding that occurs following 12 months of amenorrhoea is considered to be PMB. The incidence of PMB varies depending on the characteristics of the population studied, time elapsed since menopause and the methodology used to investigate the symptoms. In the Women's Health Initiative (WHI) randomised controlled trial, which was conducted to assess the risks and benefits of oestrogen plus progestins in postmenopausal women, 548 (6.7%) of patients in the placebo group experienced irregular vaginal bleeding [79]. Among 119 women in the placebo group participating in the Postmenopausal Estrogen/Progestin Intervention (PEPI) trial, 8.4% underwent endometrial biopsies to investigate PMB [80]. In a questionnaire-based study of 271 Danish postmenopausal women, the authors reported that 10.7% of the patients experienced spontaneous vaginal bleeding [81]. The incidence of vaginal bleeding is higher in women using postmenopausal hormone therapy [82, 83]. In addition, the frequency of vaginal bleeding is higher in the early postmenopausal years and decreases subsequently [81, 84, 85].

Pathophysiology

Several mechanisms have been proposed to explain vaginal bleeding in postmenopausal women. Menopause is characterised by cessation of ovarian function and the subsequent hypoestrogenism results in atrophic changes of the genital tract. Endometrial atrophic changes result in increased intracavitary friction, microerosions of the surface epithelium, chronic inflammatory reaction and bleeding [86]. Endometrial polyps are localised hyperplastic growths of the endometrial glands and stroma around a vascular core [87]. They can sustain intermittent torsion of the vascular pedicle, which leads to ischaemia at the apical

portion of the polyp [86]. This often results in tissue necrosis involving part of the vessel wall and subsequent irregular bleeding. The pathophysiology of abnormal vaginal bleeding in patients with uterine fibroids is less clear. Ulcerations of the surface epithelium and capillary fragility as result of the stretching of the endometrium, impaired haemostasis, rupture of blood vessels on the surface of the fibroid, dysregulation in a number of growth factors and impairment of uterine contractility have been all proposed as potential mechanisms [86, 88, 89]. Hickey et al suggested combined oestrogen and progestin hormone therapy causes changes in the endometrial structure and function, abnormal angiogenesis, vascular fragility and breakdown and altered haemostasis [90]. Tissue necrosis, vascular breakdown due to hypoxia and neovascular fragility are some of the proposed mechanisms behind vaginal bleeding in patients with endometrial cancer [86].

Diagnostic strategies

Women presenting with PMB should undergo investigations to exclude malignancy. Endometrial cancer is the most common malignancy diagnosed in women with PMB. Cervical and vulval cancers are less common causes of vaginal bleeding in postmenopausal women and can be excluded during clinical examination.

Hysteroscopy

Hysteroscopy involves direct visualisation of the uterine cavity and endocervical canal and remains the reference standard in the investigation of abnormal uterine bleeding. Hysteroscopy should be combined with directed biopsies or curettage of the endometrial cavity to improve diagnostic accuracy. This was illustrated in a study of 1286 women investigated for irregular premenopausal bleeding and PMB; hysteroscopy alone missed 34.5% of endometrial carcinoma cases [91]. Outpatient hysteroscopy offers an alternative to daycase hysteroscopy and is associated with quicker recovery, high success rate of completion and comparable patient satisfaction [92, 93]. In 2002, Clark et al published a

systematic review evaluating the accuracy of hysteroscopy in endometrial cancer diagnosis [94]. For postmenopausal women, the pretest probability of endometrial cancer was 11%, increasing to 60.9% (95% CI 50.1 – 71.1%) in patients with positive findings at hysteroscopy [94]. The probability of cancer decreased to 0.5% (95% CI 0.4 – 0.8%) with a negative test result [94].

Dilatation and curettage

Dilatation and curettage was traditionally the method of choice in evaluating women with PMB [95]. However, it is associated with significant pain, requiring use of general anaesthesia. In addition, there are concerns that the samples obtained represent only a small surface area of the endometrial cavity [95]. Moreover, there are no robust data on its accuracy in diagnosing endometrial cancer. Hence, the use of dilatation and curettage alone to investigate women PMB has been abandoned.

Office-based endometrial biopsy

Various sampling devices have been developed that allow endometrial assessment in the outpatient setting [96-101]. A meta-analysis of studies investigating the accuracy of endometrial sampling devices reported a sensitivity of 95% and specificity of 99.5% in detecting endometrial cancer in postmenopausal women [102]. The estimated sensitivity for the Pipelle device was 99.5%, higher than the other sampling devices evaluated. The failure rate to obtain an endometrial sample using an office-based device ranged from 0% to 54%. In a second meta-analysis, Clark et al reported that the failure rate for outpatient biopsies was 7% (95% CI 5%-8%) [103]. For postmenopausal women, the authors estimated that for a pretest probability of 6.9%, the posttest probability of endometrial cancer increases to 83.1% with a positive result and decreases to 1% with a negative test result [103]. Individual studies assessing the accuracy of office-based sampling devices in patients with diagnosed endometrial cancer report higher false negative results for these techniques. Zorlu et al found

2 of 26 (7.6%) cases of endometrial cancer were missed in patients that underwent Pipelle biopsy prior to hysterectomy [104]. In a similar study of 37 women diagnosed with endometrial cancer, Pipelle biopsy failed to demonstrate an accurate result in 12 (33%) cases [105]. Tumours localised to a polyp or small area of the endometrium may go undetected with office-based sampling devices [106, 107]. This was illustrated in a study of 65 postmenopausal women with known endometrial cancer that underwent Pipelle biopsy prior to hysterectomy [107]. 11 (17%) patients had a false negative Pipelle biopsy result; in 5 of these cases the tumour was confined to a polyp. In 7 of the 11 patients with a missed diagnosis the tumour was localised to ≤ 25% of the surface area of the endometrium.

Transvaginal ultrasonography

a. Endometrial thickness measurement

The technique involves the visualisation of the uterus in the sagittal plane and endometrial thickness is measured at its thickest point from one basalis layer to the other [108]. Fluid within the endometrial cavity is excluded from the measurements. In postmenopausal women, atrophic changes of the endometrium can correlate with endometrial thickness findings on transvaginal ultrasonography (TVUS) [109-111]. Endometrial thickness measurement using TVUS has high negative predictive value for endometrial cancer in women investigated for PMB [108, 112-114].

In 1998, a meta-analysis of 35 studies including 5892 women with PMB aimed to estimate the diagnostic accuracy of different endometrial thickness thresholds on TVUS in detecting endometrial cancer [115]. Studies of women using postmenopausal hormone therapy were included in the meta-analysis. The authors estimated the sensitivity and specificity for endometrial cancer at an endometrial thickness threshold of 5mm was 96% and 61%, respectively. The pretest probability of endometrial cancer decreased by 90% following an

endometrial thickness measurement less than 5mm. The authors concluded that the diagnostic performance of TVUS is optimal at an endometrial thickness threshold of 5 mm.

A subsequent meta-analysis by Gupta et al included data of 9031 patients from 57 studies [116]. The authors found the majority of the primary studies included were of poor quality. Using data from 4 studies that had the best methodology, the authors estimated the risk of endometrial cancer in patients with an endometrial thickness measurement of less than or equal to 5 mm on ultrasonography was 2.5% (95% CI 0.9%-6.4%).

Another meta-analysis included data from 9 studies on women with PMB that underwent assessment of the endometrial thickness using TVUS [117]. A questionnaire for supplementary data was sent to the authors of the primary studies. 2773 postmenopausal women without cancer and 323 women diagnosed with endometrial cancer were included; histological assessment of the endometrium was available for all cases. Endometrial thickness threshold was defined as the median value derived from the measurements in women without cancer. At this endometrial thickness threshold, the detection rate of endometrial cancer for a false positive rate of 50% and 10% was 96% (95% CI 93%-98%) and 61% (95% CI 56%-67%), respectively. The authors of this study concluded that even in the best-case scenario, 4% of endometrial cancer cases will be missed on TVUS.

A more recent meta-analysis by Timmermans et al estimated the performance of TVUS in women with PMB using individual patient data provided by the authors of the primary studies [118]. A total of 2896 women were included, of which 259 patients were diagnosed with endometrial cancer. The authors found the sensitivity and specificity of TVUS at an endometrial thickness threshold of 5 mm was lower than previously reported (90.3% and 54%, respectively). When a cut-off value of 3 mm was used, the sensitivity of TVUS to diagnose endometrial cancer was 97.9%. For a 10% pretest probability, the risk of

endometrial cancer decreases to 0.6% for a patient with an endometrial thickness measurement less than 3 mm.

b. Additional characteristics on ultrasonography

It has been suggested that predictive performance of endometrial thickness measurement to investigate women with PMB can be improved by incorporating additional ultrasound scan characteristics [119-122].

Several studies have shown that heterogeneity or heterogenous echogenicity of the endometrium are highly predictive of endometrial carcinoma [119, 123-125]. Other features shown to have a correlation with endometrial cancer include increased endometrial echogenicity and presence of irregular endometrial-myometrial border [123, 125]. However, the detection of these features on ultrasonography is highly dependent on the experience of the operator and concerns about reproducing these results have been raised [119, 125].

Other authors found that use of Doppler variables has greater sensitivity than endometrial thickness measurement in predicting endometrial cancer in postmenopausal women [126-128]. In a study of 85 women presenting with premenopausal bleeding and PMB, the authors reported that Doppler assessment of the uterine arteries had 100% sensitivity for detecting endometrial hyperplasia and cancer [127]. Other authors found a correlation between the pattern of the vessels within the endometrium and endometrial cancer [129], while irregular branching of endometrial blood vessels was shown to be the best predictor of cancer in a subsequent study [125]. Contrary to these findings, multiple reports suggests that Doppler characteristics perform no better that endometrial thickness measurement alone in distinguishing between benign pathologies and endometrial cancer [124, 130-132]. Similar to assessment of endometrial morphology, reproducibility of the findings is an issue, especially for inexperienced examiners [133].

Several studies have shown that injection of normal saline within the endometrial cavity at the time of TVUS (saline contrast sonohysterography, SCH) can improve detection of endometrial lesions, including endometrial cancer [134-136]. A meta-analysis of 24 studies, reporting on 2278 SCH procedures found a 95% sensitivity and 88% specificity in evaluating the uterine cavity in patients with irregular vaginal bleeding [121]. The success rate of SCH was significantly lower in postmenopausal women compared to premenopausal (83.6% versus 93%, respectively). The posttest probability of uterine abnormalities following a negative test was 7%. The authors of this meta-analysis did not report on the diagnostic performance of the technique in distinguishing between patients with endometrial cancer from those with benign uterine pathologies. In a different study of 105 women with PMB and endometrial thickness greater than 5 mm on TVUS, SCH had 44% sensitivity in diagnosing endometrial cancer, similar to that of conventional ultrasonography [137]. The authors reported that 36% of endometrial cancer cases were not diagnosed on SCH. Other authors reported similar low sensitivity of saline contrast sonohysterography in detecting endometrial cancer [138]. It has also been suggested that SCH is more likely to fail (no distension of the uterine cavity) in cases of endometrial cancer [137, 139].

Estimation of endometrial volume using 3-dimensional ultrasonography has been found to have better accuracy than conventional ultrasonography in diagnosing endometrial cancer in postmenopausal women [140, 141]. However, although endometrial volume is significantly higher in women with endometrial cancer compared to those with benign pathologies, a volume threshold that predicts endometrial cancer has not been clearly established [122, 140-142]. Several studies have shown that evaluation of 3-dimensional power Doppler indices has excellent discriminatory ability for endometrial cancer [122, 142].

Economic evaluation of diagnostic strategies for PMB

Several studies have attempted to estimate the cost-effectiveness of diagnostic strategies for women presenting with a first episode of PMB [143-147]. These estimations are based on several assumptions, including the diagnostic performance of different strategies, patient's life expectancy and associated healthcare costs. Most studies suggest that cost-effectiveness of the investigation pathways depends on the prevalence of endometrial cancer in the population and age of the patient [143, 145-147]. A strategy with TVUS as the initial test is the most cost-effective, especially when the prevalence of endometrial cancer is low [144-147]. Medverd et al found that TVUS was a more cost-effective initial test than endometrial biopsy if the prevalence of endometrial cancer and hyperplasia was less than or equal to 31% [145]. Dijkhuizen et al estimated that TVUS was the most cost-effective strategy in populations with prevalence of endometrial cancer less than 15.3% [146].

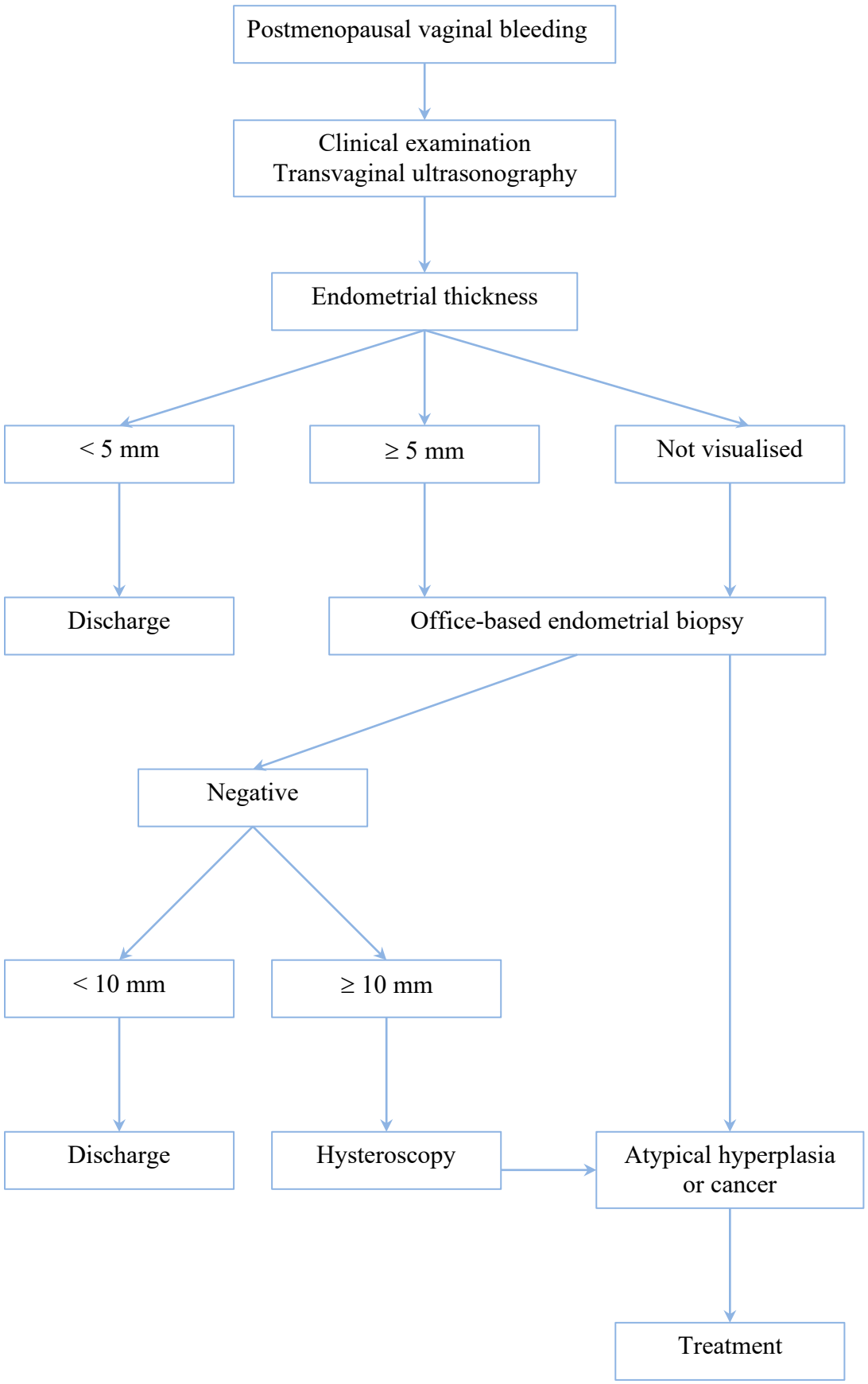
Patient preferences

There is limited understanding of patients' preferences on available diagnostic strategies to investigate PMB. Timmermans et al performed a structured interview of 39 women that had undergone hysteroscopy for investigation of PMB [148]. Only 5% of the women reported that they would accept a false negative rate of greater than 5% for a test used to investigate PMB. However, the study sample was small and comprised of women that had already undergone hysteroscopy with a negative histology result. Hence, it is difficult to extrapolate these results to patients presenting with the first episode of PMB. The authors of a different, questionnaire-based study of 207 pre- and postmenopausal women evaluated the attitudes of patients regarding TVUS [149]. The majority of patients reported that they would undergo TVUS if recommended by their doctor, with acceptability greatest amongst older women.

Current clinical evaluation

The investigation pathway for women with PMB is described in Flowchart 1. All patients with PMB are referred to secondary care for investigations under the two-week-wait pathway [150]. The initial step in assessing patients presenting with PMB is to obtain clinical history and perform physical examination. The clinical history focuses on determining the menopausal status of the patient and to confirm the bleeding is of genital tract origin. It should also elicit the presence of risk factors for developing endometrial cancer. Physical examination should aim to exclude systemic or topical causes for PMB such as bleeding disorders, vulvovaginal or cervical tumours and trauma.

TVUS is the initial test to evaluate women presenting with PMB. An endometrial thickness threshold of 5 mm is used to select the patients that will undergo an office-based biopsy.



Flowchart 1. Current management of women presenting with postmenopausal bleeding

1.3 Hypothesis and study objectives

Hypothesis: A combination of clinical characteristics and ultrasonography findings can be used to create an individualised prediction of risk of endometrial cancer in women presenting with postmenopausal vaginal bleeding (PMB).

Primary objective: To develop and internally validate diagnostic predictive models for patients investigated for PMB.

Secondary objectives:

1. To estimate the incidence and causes of PMB in the population

2. To estimate the risk of endometrial cancer is the following subgroups of women presenting with PMB:

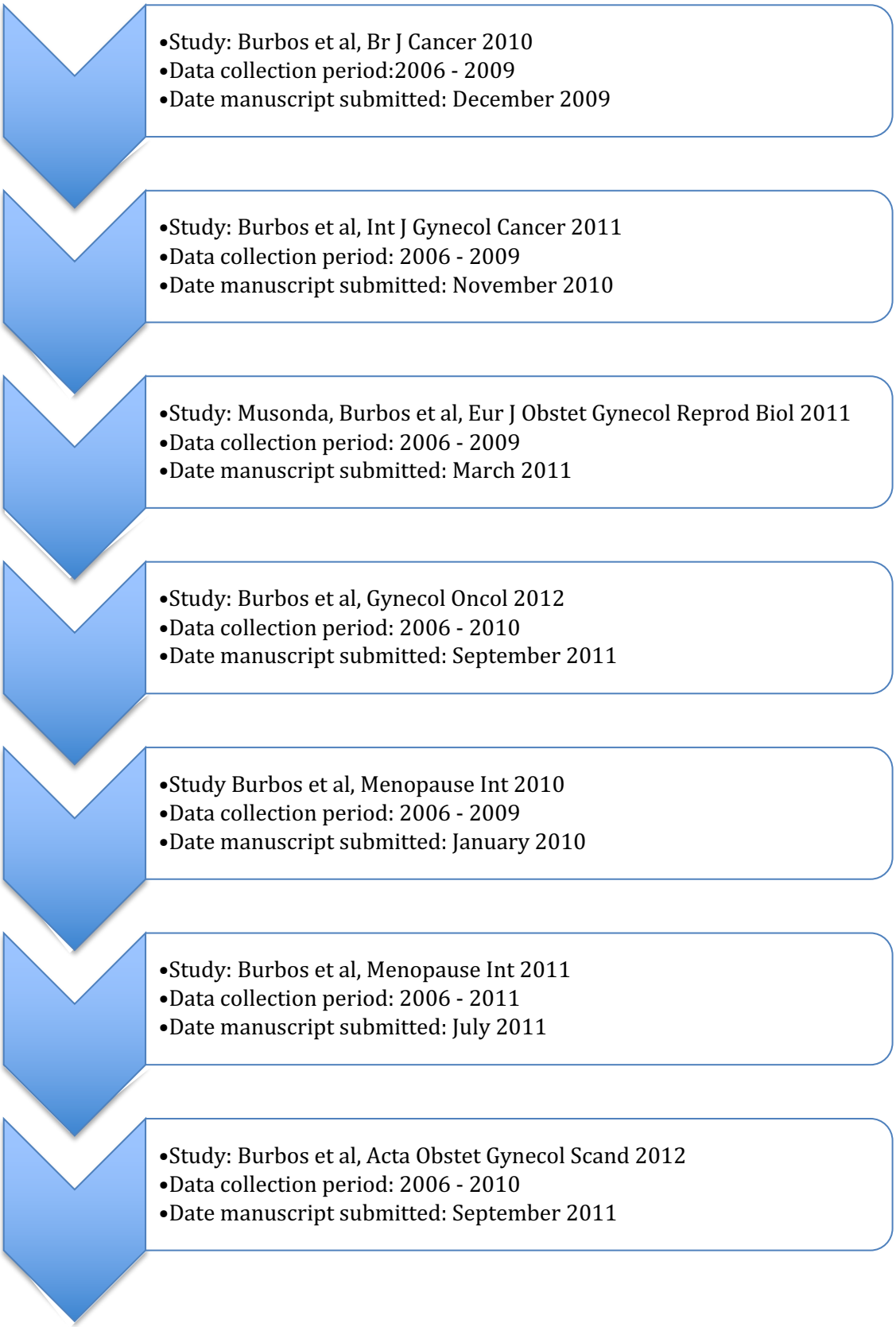
- Young postmenopausal women
- Women using postmenopausal hormone therapy
- Women with inadequate assessment of endometrium on ultrasonography

1.4 Research questions

- Can we incorporate clinical and demographic characteristics to predict the risk of endometrial cancer in women with PMB?
- What is the differential diagnosis of PMB in different age groups?
- What is the risk of endometrial cancer in the following populations:
    - Young postmenopausal women
    - Women using postmenopausal hormone therapy
    - Women with indistinct endometrium on TVUS

1.5 Timeline for publications

All the studies included in this thesis are based on data collected prospectively between 2006 and 2012. The manuscripts were submitted for publication in order of clinical and academic priority, as decided by the authors. The manuscripts are presented according to the primary and secondary objectives of the thesis rather than by chronological order of publication. A graphic summary of the timeline of the publications is shown in Flowchart 2.



Flowchart 2. Timeline for publications

Chapter 2. Predicting the risk of endometrial cancer in postmenopausal women presenting with vaginal bleeding: The Norwich DEFAB risk assessment tool.

2.1 Introduction

Clinical prediction models use a combination of predictors based on clinical findings, medical history and test results to estimate the absolute risk or probability of an outcome [151]. Several predictive models have been developed to predict the risk of endometrial cancer in women presenting with postmenopausal vaginal bleeding (PMB). In this chapter, I present a literature review of studies on predictive models for women with PMB. In addition, I present the development of a new diagnostic predictive model based on data from a prospective study. The aim of the new model is to improve the ability of clinicians to stratify the risk of endometrial cancer for patients presenting with PMB, in order to prioritise diagnostic tests.

2.2 Literature review

Available predictive models for women presenting with PMB use a combination of clinical characteristics and/or findings on ultrasonography to predict the risk of endometrial cancer. There are several deficiencies observed in the development of these models.

Feldman et al performed a nested case-control study including a total of 203 women presenting with irregular vaginal bleeding [152]. The data were extracted retrospectively from a pathology database. Patients aged older than 49 years, that underwent an endometrial biopsy or dilatation and curettage, were included. Cases were defined as patients diagnosed with endometrial carcinoma and complex hyperplasia of the endometrium, with or without atypia. The study group included 150 postmenopausal patients presenting with vaginal bleeding. 36 (24%) cases of endometrial cancer were diagnosed in the postmenopausal group. The authors developed a model to predict the risk of endometrial cancer and complex

endometrial hyperplasia (combined) using 4 clinical variables: age 70 or over, diabetes, hypertension and menopausal status. The risk of endometrial cancer/complex hyperplasia was estimated to be 87% if all the risk factors were present in an individual, decreasing to 2.6% if none of the factors was present. For postmenopausal patients, if there were no risk factors present, the background risk of endometrial cancer was 5%. On a multivariate analysis including only postmenopausal patients, a history of diabetes was not associated with a statistically significant increase in the risk of endometrial cancer. In the postmenopausal group of patients, the following factors were found to be statistically significant for endometrial cancer diagnosis: age 70 or over (OR = 16, p <0.0001), nulliparity (OR = 2.8, p =0.03) and a history of non-breast cancer (OR =6.6, p =0.009). The authors of this study were the first to investigate the correlation between bleeding pattern (frequency and volume) and risk of diagnosing endometrial cancer. The main limitations are related to the retrospective nature of the study, small number of endometrial cancer cases and the inclusion of premenopausal women in the analysis.

Weber et al performed a prospective study of 159 women presenting with PMB [153]. Women on postmenopausal hormone therapy were excluded from the study. All patients underwent a transvaginal ultrasonography (TVUS) and the following sonographic parameters were evaluated: size of the uterus, endometrial thickness, endometrial morphology/border and presence of intrauterine fluid. All patients underwent histological evaluation of the endometrium. 62 (39%) patients were diagnosed with endometrial cancer. 26 (16%) patients were found to have endometrial hyperplasia or polyps and their histology results were classified as suspicious. The authors combined patients diagnosed with cancer and those with suspicious histopathology results in the same outcome group, named pathological. The authors identified three variables that can predict pathological findings: endometrial thickness measurement (less than or equal to [≤] 5 mm or greater than [>] 5 mm), endometrial border

appearance (regular, irregular) and endometrial morphology (homogenous, heterogeneous). For patients with endometrial thickness >5 mm, irregular endometrial border and heterogeneous endometrium on TVUS, the likelihood of endometrial cancer or suspicious pathology was 80%. In cases where endometrial thickness measured <5 mm, endometrial border was regular and homogenous endometrial morphology was found on TVUS, no cases of cancer were diagnosed and risk of suspicious pathology was 6%. Due to study design, the model presented is aimed at predicting the risk of endometrial pathology including cancer, hyperplasia and polyps. Interestingly, the prevalence of endometrial cancer in the study cohort is higher than the average observed in women with PMB and may reflect selection bias.

Weber et al performed a retrospective case control study of women presenting with irregular premenopausal bleeding and PMB [154] . The study included 57 cases (endometrial cancer and hyperplasia) and 137 controls. Only 15 patients were diagnosed with endometrial cancer. Among patients with endometrial hyperplasia, 79% had no histological atypia. The authors used developed two models using several clinical characteristics as predictors. The first model combined patient's age, menopausal status, a history of diabetes, hypertension, patient's weight (in kilograms), parity and use of postmenopausal hormone therapy. It had a discriminatory accuracy as described by the area under receiver operating characteristic curve (ROC) equal to 0.75 ($p < 0.001$). The second model included only patient's age, a history of diabetes, the patient's weight and parity. This model had an area under ROC curve equal to 0.74 ($p < 0.001$). There was no difference in the predictive ability between these two models ($p = 0.514$). The main issue with this work is related to the study design. Case-control studies are not ideal for prognostic analysis, as they do not allow estimation of absolute risks [151]. In addition, the study population was not clearly defined and included a heterogeneous group of premenopausal and postmenopausal women.

Randelzhofer et al evaluated 321 consecutive patients referred with PMB to a tertiary clinic using TVUS [155]. All patients underwent dilatation and curettage to obtain an endometrial sample for histological examination. Patients using postmenopausal hormone therapy or tamoxifen were excluded from the study. The authors evaluated the role of ultrasound scan variables in predicting the risk of endometrial cancer. 95 (29.6%) patients were diagnosed with endometrial cancer. The authors found that heterogeneous endometrial structure, irregular myometrial border and endometrial thickness >10 mm were the strongest predictors of cancer in the study population. The estimated probability of endometrial malignancy was 94.2% if all the above features were detected on TVUS. The risk of endometrial cancer for patients with endometrial thickness measurement <10 mm, smooth myometrial border and homogeneous endometrial structure, was 2.8%. The sensitivity, specificity, positive and negative predictive value of the model developed was 96.8%, 61.9%, 52% and 98%, respectively. However, this model was derived in a highly selected population of patients with PMB and its predictive performance may be reduced when applied in a different setting.

Bachman et al analysed data from a cohort of 428 patients attending an ambulatory hysteroscopy clinic for investigation of PMB [156]. 154 patients were using postmenopausal hormone therapy. All patients underwent TVUS, hysteroscopic assessment of the endometrium and endometrial biopsies. 19 (4.4%) patients were diagnosed with endometrial cancer. The authors developed and evaluated four predictive models. The first model combined two clinical characteristics, patient's age and use of postmenopausal hormone therapy only. The second model incorporated the above clinical characteristics and endometrial thickness measurement on ultrasonography (endometrial thickness cut-off value of 5 mm). The third predictive model included clinical characteristics and hysteroscopic findings (normal or suspicious). Finally, the fourth model combined clinical characteristics, endometrial thickness measurement and hysteroscopic findings. The area under ROC curve

for each of the above models in predicting endometrial disease (endometrial cancer and hyperplasia combined) was 0.8, 0.82, 0.910 and 0.914, respectively. The authors estimated that the risk of endometrial cancer for women younger than 60 years, using postmenopausal hormone therapy, was 0.2%. The risk of endometrial cancer increased to 12.9% in women older than 60 years that did not use postmenopausal hormone therapy. If TVUS or hysteroscopy findings were positive in women over the age of 60 years that did not use postmenopausal hormone therapy, the risk of endometrial cancer was 16.2% and 59.4%, respectively. If both tests were positive, the estimated risk of cancer was 68.6%. One of the concerns with this study is the small sample size used to derive the predictive models. The number of outcome events (endometrial cancer) is too small compared to the number of predictors evaluated. In addition, the authors chose to include only two clinical characteristics in the predictive models, and other variables such as body mass index, a history of diabetes, hypertension and use of tamoxifen were not evaluated.

Bruchim et al conducted of a study including 95 women presenting with PMB, of which 9 (9.5%) were diagnosed with endometrial cancer [157]. None of the patients used postmenopausal hormone therapy. All patients underwent TVUS for measurement of the endometrial thickness and endometrial biopsies. Time elapsed since menopause and endometrial thickness measurement were the only predictors evaluated. The authors derived a formula to calculate the probability of endometrial cancer based on the measurement of endometrial thickness on TVUS and the age of the patient. Odds ratios for endometrial cancer were shown graphically. The study is limited by its small sample size, as well as the authors not considering other risk factors as predictors for endometrial cancer.

Opmer et al reported the results of a multicentre, prospective cohort study including 540 women presenting with PMB [158]. Patients using postmenopausal hormone therapy were excluded from the study. All patients underwent a TVUS to measure endometrial thickness.

An endometrial biopsy was performed only if endometrial thickness measurement was greater than 4 mm. 56 (10.3%) patients diagnosed with endometrial cancer and 9 (1.7%) patients with atypical hyperplasia were included in the same outcome group. The authors developed two predictive models. The first model was based on clinical characteristics and had an area under ROC curve value of 0.76 (95% CI 0.71 – 0.82). The selected predictors were age, body mass index, diabetes, parity and use of anticoagulants. A second predictive model that incorporated patient's characteristics and endometrial thickness measurement, had an area under the ROC curve of 0.90 (95% CI 0.87 – 0.93). The authors evaluated three diagnostic strategies for women presenting with PMB. They found that an approach where all women undergo an initial TVUS and subsequently the risk of endometrial cancer/hyperplasia is estimated by combining clinical characteristics and endometrial thickness measurement to determine further investigations, had the best diagnostic accuracy (area under ROC curve 0.90). This is a well-designed study, however the main limitation is the small sample size.

Opolskiene et al evaluated 120 women with PMB using grey scale and power Doppler ultrasound [125]. Patients found to have an endometrial thickness measurement ≥4.5 mm were included in the study. The authors excluded patients with incomplete TVUS examination of the endometrium and patients with fluid within the endometrial cavity. Hysteroscopy or dilatation and curettage were defined as reference tests. 30 (25%) patients were diagnosed with endometrial cancer. Clinical characteristics evaluated included patient's age and use of postmenopausal hormone therapy. However, the predictive model developed was based on TVUS variables only. The proposed model incorporated endometrial thickness measurement (optimal cut-off value of 15 mm) and heterogeneous echogenicity of the endometrium. The above model had a discriminatory ability estimated by the area under the ROC curve of 0.91. The discriminatory ability of the model did not change significantly

when Doppler imaging characteristics were incorporated. The development of this model was based on a small sample size and highly selective population of patients with PMB.

2.2 What does this study add?

This is the first study that used an adequate sample size for the development of a diagnostic predictive model for patients presenting with PMB. The aim of the predictive model is to improve risk stratification of patients with PMB and to prioritise diagnostic tests accordingly.

2.4 What went well?

I conceived the idea for this study while observing clinical practice in my department. After performing a literature review, I established the need for developing a new predictive model for women presenting with PMB. While conducting this study, I improved my knowledge in database development and statistical methods used in research. I also gained better understanding of ethical considerations in research studies. This work helped me to better understand the importance of collaboration in research and the valuable experience that each member of the group brings to the project. I also gained an appreciation of the value of adhering to set timelines. The article was published in a high impact factor journal and this motivated me to pursue research further.

2.5 What could have been done differently?

The statistician was involved at an early stage in this research project but not at its outset. Involvement of the statistician at an earlier stage is likely to have helped me with the database design and more efficient data collection.

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Full Paper

Predicting the risk of endometrial cancer in postmenopausal women presenting with vaginal bleeding: the Norwich DEFAB risk assessment tool

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BACKGROUND: This study aimed to show the longitudinal use of routinely collected clinical data from history and ultrasound evaluation of the endometrium in developing an algorithm to predict the risk of endometrial carcinoma for postmenopausal women presenting with vaginal bleeding.

METHODS: This prospective study collected data from 3047 women presenting with postmenopausal bleeding. Data regarding the presence of risk factors for endometrial cancer was collected and univariate and multivariate analyses were performed.

RESULTS: Age distribution ranged from 35 to 97 years with a median of 59 years. A total of 149 women (5% of total) were diagnosed with endometrial carcinoma. Women in the endometrial cancer group were significantly more likely to be older, have higher BMI, recurrent episodes of bleeding, diabetes, hypertension, or a previous history of breast cancer. An investigator best model selection approach was used to select the best predictors of cancer, and using logistic regression analysis we created a model, 'Norwich DEFAB', which is a clinical prediction rule for endometrial cancer. The calculated Norwich DEFAB score can vary from a value of 0 to 9. A Norwich DEFAB value equal to or greater than 3 has a positive predictive value (PPV) of 7.78% and negative predictive value (NPV) of 98.2%, whereas a score equal to or greater than 5 has a PPV of 11.9% and NPV of 97.8%.

CONCLUSION: The combination of clinical information with our investigation tool for women with postmenopausal vaginal bleeding allows the clinician to calculate a predicted risk of endometrial malignancy and prioritise subsequent clinical investigations.

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Keywords: endometrial; risk; postmenopausal; prediction; model

Postmenopausal bleeding refers to any genital tract bleeding in a postmenopausal woman, other than the expected bleeding that occurs in women taking sequential hormone replacement therapy (HRT). Because postmenopausal bleeding is the most common symptom of endometrial cancer, when postmenopausal bleeding occurs, clinical evaluation is indicated (Goldstein *et al*, 2001). Approximately 10% (range 1 – 25%) of women presenting with postmenopausal bleeding will be diagnosed with endometrial carcinoma (Gambrell *et al*, 1978; Alberico *et al*, 1989; Iatrakis *et al*, 1997). Endometrial atrophy is the most common cause of genital tract bleeding among postmenopausal women (Iatrakis *et al*, 1997). Endometrial hyperplasia and polyps are also common causes.

Two different forms of endometrial carcinoma have been identified. Type-I cancers have an endometrioid histology and account for 70 – 80% of endometrial carcinomas. They are associated with unopposed oestrogen stimulation of the endometrium and tend to arise in women with obesity, hyperlipidaemia, and other hyper-oestrogenic conditions. Type-II cancers have a non-endometrioid histology and arise in women who are less likely to have the clinical associations seen in type-I cancers (Bokhman, 1983). Several risk factors such as obesity, tamoxifen use, increasing age, hypertension, diabetes, and unopposed use of exogenous oestrogens are strongly associated with increased risk of type-I endometrial cancer (Persson *et al*, 1989; Soler *et al*, 1999; Cohen, 2004; Lachance *et al*, 2006; Friberg *et al*, 2007; Lucenteforte *et al*, 2007; Renehan *et al*, 2008). Early menarche and late menopause have also been implicated due to prolonged oestrogen stimulation of the endometrium. Nulliparity as an isolated risk factor does not appear to increase the risk of endometrial cancer, although due to the high frequency of anovulatory cycles there may be an association in women with subfertility (Chen and Berek, 2008). Hereditary non-polyposis colorectal cancer is a significant but rare risk factor, with descendants of an affected family member carrying a theoretical 50% lifetime risk of endometrial cancer (Aarnio *et al*, 1995).

Currently, controversy exists as to whether transvaginal ultrasonography or endometrial biopsy should be used as the initial diagnostic step for clinical evaluation of women presenting with postmenopausal bleeding (Goldstein *et al*, 2001). In addition, decisions made about the most appropriate investigations that need to be performed, are not always guided by clinical history. The few studies that attempt to include information gained from

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clinical history to predict the risk of endometrial carcinoma are too small to develop a predictive model (Weber *et al*, 1999; Bachmann *et al*, 2003).

The aim of our study was to use routinely collected clinical data from history and ultrasound evaluation of the endometrium to develop an algorithm to predict the risk of endometrial carcinoma in women presenting to secondary care with postmenopausal vaginal bleeding.

MATERIALS AND METHODS

Participants

This is a prospective cohort study, conducted at a gynaecological oncology centre in the United Kingdom, between February 2006 and May 2009. All postmenopausal women presenting with vaginal bleeding to the postmenopausal bleeding clinic were included. Menopause was defined as at least 12 months of spontaneous amenorrhoea. Premenopausal women were not included in the study as there is no standard threshold for endometrial thickness in this group that is considered abnormal. Other groups of women seen at the clinic that were excluded from the study included asymptomatic women with an incidental finding of increased endometrial thickness on imaging and asymptomatic women with abnormal endometrial cytology found on cervical smear.

Procedures

All women presenting with vaginal bleeding underwent transvaginal ultrasound scanning to evaluate the endometrium. The double-wall endometrial thickness was measured in an anterio-posterior dimension from one basalis layer to the other. In keeping with departmental guidelines, when endometrial thickness was measured to be less than 5 mm no further investigations were performed as evidence suggests a low probability of cancer below this threshold (Karlsson *et al*, 1995; Smith-Bindman *et al*, 1998). For the purpose of the study, we considered all women with endometrial thickness less than 5 mm as negative for endometrial cancer.

Women with endometrial thickness equal to or greater than 5 mm had endometrial sampling performed using an endometrial Pipelle device. Hysteroscopic evaluation of the endometrium with biopsy under a general anaesthetic was performed if Pipelle biopsy was not possible or did not yield sufficient tissue for histological diagnosis. A hysteroscopy was also performed for any woman re-appearing at the clinic for a second time with a recurrent episode of bleeding.

Clinical risk factors – data collection

The clinic collects routine data regarding essential clinical information and presence of risk factors for endometrial cancer using a pre-designed proforma. Data extracted from these forms for this study were age of the patient at presentation, body mass index (BMI), use of HRT, presence of hypertension and diabetes, previous history of breast cancer, and use of tamoxifen. Endometrial thickness measured on ultrasound scan and results of histology when performed were also recorded. We excluded data regarding parity as we consider that it is the frequency of anovulatory cycles that increases the risk of endometrial cancer and not nulliparity *per se*. Data from 90% of the patients were collected prospectively and only in 10% of the cases was it collected retrospectively.

We also attempted to assess whether the bleeding pattern of women had any predictive value in the histological outcome. The amount of bleeding was characterised as spotting, light (=less than a period), and heavy (=like a period or worse). Any event lasting less than 7 days was defined as a single bleeding episode. Recurrent episodes were defined as any bleeding episode lasting 7 or more days or two or more separate bleeding events within the last 12 months.

All the data analysed were collected as part of the routine investigations and treatment. The patients were investigated according to established evidence-based departmental guidelines.

Statistical analysis

The distributions of continuous variables were not symmetric. To test for normality, the Shapiro-Wilk W-test was used, as was the q-q plot to investigate normality graphically (results not shown). There was no evidence to suggest that data were normally distributed, hence in the descriptive statistics for continuous variables, we report median and inter-quartile range. To avoid inflating the type-I error rate, loss of power, residual confounding, and bias, continuous predictor variables were not categorised (Del Priore *et al*, 1997; Austin and Brunner, 2004; Royston *et al*, 2006). To test any differences we used a non-parametric Wilcoxon rank sum (Mann–Whitney) test. Binomial exact methods were used to calculate 95% confidence intervals (CIs) of the proportions and to test any differences in the proportions observed. χ^2-test was used after checking the expected assumptions. An investigator best model selection approach was used to select the best predictors of cancer in the multiple logistic regression model as opposed to machine-led step-wise regression, which is not advisable (Hurvich and Tsai, 1990; Derksen and Keselman, 1992). Selection of predictor variables was performed by using the likelihood ratio test after estimation of the nested models by adding and eliminating variables one at a time. The likelihood ratio test is similar to using model selection indices such as Akaike information criterion (AIC) or Bayesian information criterion (BIC). All analyses were performed using STATA software, version 10.1 SE (Stata Corporation, College Station, TX, USA).

RESULTS

Demographics

During a 39-month interval, 3047 women were investigated for postmenopausal vaginal bleeding. Age distribution ranged from 35 to 97 years with a median of 59 years. A total of 149 women (5% of total) were diagnosed with endometrial carcinoma. Women with all types of endometrial cancer were included in this group. The remaining 2898 women (95%) were included in the non-cancer group for the purposes of the study.

Clinical risk factors

The results of univariate analysis to assess for correlation between individual clinical characteristics and development of endometrial cancer are given in Table 1. Women in the endometrial cancer group were significantly older (median 64 *vs* 59 years; $P<0.0001$) and had higher BMI (31 *vs* 28 kg m^{-2}, $P<0.0001$) than women without cancer. They were more likely to have diabetes ($P<0.0001$) and hypertension ($P=0.001$). The duration of use of HRT did not appear to increase the risk of endometrial cancer ($P=0.243$). The women in the endometrial cancer group were significantly more likely to have a previous history of breast cancer ($P=0.025$). However, the duration of use of tamoxifen in the breast cancer group did not appear to increase the risk of endometrial cancer ($P=0.091$). The amount of vaginal bleeding did not appear to be associated with increased risk of endometrial cancer ($P=0.289$). Recurrent episodes of vaginal bleeding were significantly more likely to be associated with endometrial cancer than a single bleeding event ($P<0.0001$). Endometrial thickness on ultrasound scan was significantly higher in women with endometrial cancer (14.9 *vs* 4.6 mm; $P<0.0001$).

Table 1 Basic characteristics of the population. Univariate comparison

Factors	Cancer, $n = 149$ (5%)	No cancer, $n = 2898$ (95%)	*P*-value
Age (years)	64 (59–72)	59 (54–67)	<0.0001[a]
BMI (kg m^{-2})	31 (27–36)	28 (25–32)	<0.0001[a]
Diabetes			
Yes	25 (17%, 11–24%)	158 (5%, 5–6%)	<0.0001[b]
No	124 (83%, 76–89%)	2740 (95%, 94–95%)	
Hypertension			
Yes	56 (38%, 30–46%)	741 (26%, 24–27%)	0.001[b]
No	93 (62%, 54–70%)	2157 (74%, 73–76%)	
HRT duration (years)	9 (4–20)	5 (2–10)	0.243[a]
Breast cancer			
Yes	16 (11%, 6–17%)	178 (6%, 5–7%)	0.025[b]
No	133 (89%, 83–94%)	2720 (94%, 93–95%)	
Tamoxifen use (years)	4.5 (2–8)	3 (2–5)	0.091[a]
*Amount of bleeding**			
Spotting	39 (27%, 20–35%)	611 (21%, 20–23%)	
Light	80 (55%, 46–63%)	1620 (57%, 55–59%)	0.289[b]
Heavy	27 (18%, 13–26%)	614 (22%, 20–23%)	
*Frequency of bleeding**			
Single	36 (24%, 18–32%)	1541 (53%, 52–55%)	<0.0001[b]
Recurrent	112 (76%, 68–82%)	1345 (47%, 45–48%)	
Endometrial thickness (mm)	14.9 (11.0–21.0)	4.6 (3.0–7.8)	<0.0001[a]

Abbreviations: BMI = body mass index; HRT = hormone replacement therapy. Values are median (inter-quartile range), number (percent, 95% CI of percent).
[a]Two-sample Wilcoxon rank sum test (Mann–Whitney test). [b]χ^2-Test. *Percentages worked on less numbers from the overall due to missing values.

Table 2 Adjusted predictors of cancer (odds ratio) from the best model that fits the data well

Predictors of cancer	Odds ratio (95% confidence interval)	*P*-value
Age (years)	1.04 (1.02–1.06)	<0.0001
BMI (kg m^{-2})	1.03 (1.00–1.06)	0.038
Endometrial thickness (mm)	1.15 (1.13–1.18)	<0.0001
Frequency of bleeding		
Single episode	1	
Recurrent episode	3.93 (2.48–6.23)	<0.0001
Diabetes		
Yes	1.92 (1.07–3.45)	0.030
No	1	

Abbreviation: BMI = body mass index.

Table 3 Overall sensitivity, specificity, and correct classification for each DEFAB cut-off point

Cut-point	Sensitivity	Specificity	Correctly classified	LR (+)	LR (−)	d-OR
(>=0)	100.00%	0.00%	4.88%	1.000		—
(>=1)	95.95%	25.57%	29.00%	1.289	0.159	8.11
(>=2)	88.51%	44.70%	46.84%	1.601	0.257	6.23
(>=3)	81.76%	50.28%	51.81%	1.644	0.363	4.53
(>=4)	79.05%	52.56%	53.86%	1.667	0.399	4.18
(>=5)	67.57%	74.43%	74.09%	2.642	0.436	6.06
(>=6)	43.24%	92.38%	89.98%	5.673	0.614	9.24
(>=7)	16.22%	96.92%	92.98%	5.258	0.865	6.08
(>=8)	8.78%	99.27%	94.86%	12.071	0.919	13.13
(>=9)	3.38%	99.90%	95.19%	32.510	0.967	33.62
(>9)	0%	100.0%	95.12%		1.000	—

ROC Area = 0.769, 95% CI (0.730–809). LR (+) = Likelihood ratio (+ve) = Pr (+ve|+ve)/Pr (+ve|−ve). LR (−) = Likelihood ratio (−ve) = Pr (−ve|+ve)/Pr (−ve|−ve). d-OR = diagnostic odds ratio = LR (+)/LR (−).

As a result of the statistical analysis the investigating team determined that the factors considered best predictors of endometrial malignancy were age, BMI, presence of diabetes, and endometrial thickness (*P*-value <0.0001, 0.038, 0.030, and <0.0001, respectively). Recurrent episodes of vaginal bleeding were significantly more likely to be associated with endometrial cancer than a single episode (odds ratio 3.93, 95% CI 2.48–6.23), taking into account diabetic status, age, BMI, and endometrial thickness (Table 2).

Predictive model: Norwich DEFAB

We have created a model with regard to predicting the odds of endometrial carcinoma in postmenopausal women presenting with vaginal bleeding. We are calling this tool DEFAB, which is a clinical prediction rule based on Diabetes, Endometrial thickness, Frequency of bleeding, Age, and BMI. In the DEFAB criteria, presence of diabetes in a patient scores 2; endometrial thickness $\geqslant$14 mm scores 1; recurrent episodes of bleeding scores 4; age $\geqslant$64 years scores 1; and BMI $\geqslant$ 31 kg m^{-2} scores 1. If a criterion is absent, then the score is 0. The calculated Norwich DEFAB score can vary from a value of 0–9. The scores were arrived at by taking account of the predictive odds of cancer from the adjusted model.

The overall sensitivity, specificity, and likelihood ratio for each Norwich DEFAB cut-off point are shown in Table 3. Table 3 also shows the overall proportion (percentage) of the total numbers that have been correctly classified by Norwich DEFAB in each category. The difference in the odds for malignancy predicted by a Norwich DEFAB value equal to or greater than 3 and equal to or greater than 5 was 4.53 and 6.06, respectively. Table 4 shows the

Table 4 Sensitivity, specificity, PPV, and NPV for DEFAB cut-offs of ⩾3 and ⩾5

	DEFAB score ⩾3, estimate (95% CI)	DEFAB score ⩾5, estimate (95% CI)
Sensitivity	81.9% (74.7–87.7%)	67.8% (59.6–75.2%)
Specificity	50.1% (48.2–51.9%)	74.1 (72.5–75.7%)
ROC area	0.660 (0.627–0.692)	0.710 (0.671–0.748)
PPV	7.78% (6.50–9.21%)	11.9% (9.77–14.2%)
NPV	98.2% (97.4–98.8%)	97.8% (97.1–98.4%)

Abbreviations: CI = confidence interval; NPV = negative predictive value; PPV = positive predictive value; ROC = receiver operating characteristic curve.

sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and receiver operating characteristics (ROC) area for Norwich DEFAB cut-off values equal to or greater than 3 and 5. A Norwich DEFAB value equal to or greater than 3 achieved a sensitivity of 81.9% (95% CI, 74.7–87.7%), specificity of 50.1% (95% CI, 48.2–51.9%), and an ROC area of 0.660 (95% CI, 0.627–0.692). For a Norwich DEFAB cut-off score equal to or greater than 5, sensitivity, specificity, and ROC area were 67.8% (95% CI, 59.6–75.2%), 74.1% (95% CI, 72.5–75.7%), and 0.710 (95% CI, 0.671–0.748), respectively.

The accuracy of a test depends on how well the test separates the group being tested into those with and without the disease in question. The area under the ROC curve measures accuracy. An area of 1 represents a perfect test and an area of 0.5 represents a worthless test. The overall predictive ability for the Norwich DEFAB measured by the area under the ROC curve was 0.7694 (Figure 1). Our clinical prediction rule would be considered to be of 'fair accuracy' at separating women with cancer from women without cancer, according to the traditional academic point system: fail, poor, fair, good, excellent.

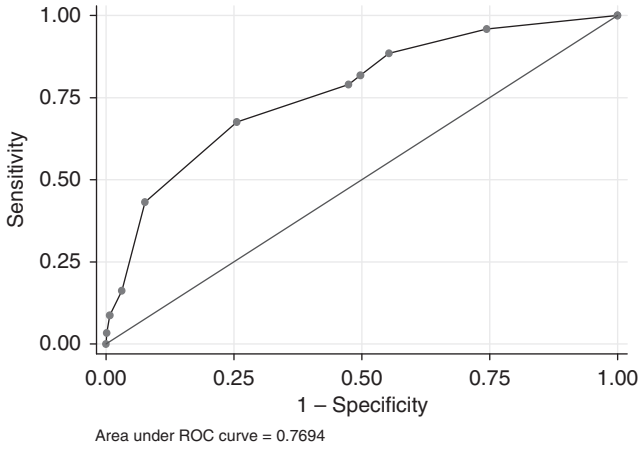

Figure 1 Area under the ROC curve for DEFAB scores.

DISCUSSION

The main objective of the diagnostic evaluation of women with postmenopausal vaginal bleeding is exclusion of malignancy. Women with postmenopausal uterine bleeding may be assessed initially with either endometrial biopsy or transvaginal ultrasonography. Initial evaluation does not require performance of both tests (ACOG Committee Opinion No 440, 2009). Currently, with respect to mortality, morbidity, and quality-of-life end points, there are insufficient data to comment as to whether transvaginal ultrasonography or endometrial biopsy is most effective for initial evaluation of this group of women. Which approach is used initially depends on the risk of the patient and the nature of the clinician's practice (Goldstein *et al*, 2001). As it is not clear which approach for evaluation of the endometrium is more effective, we attempted in this study to find a way of discriminating patients at low and high risk of endometrial cancer. This individualised risk prediction will allow clinicians to make more efficient use of the available diagnostic resources and simultaneously minimise false-negative results from various investigations.

Currently, information gained from the clinical history is not taken into account when performing risk assessment for postmenopausal women with vaginal bleeding. The optimal assessment of women with postmenopausal bleeding would be to stratify the population of women into high-risk and low-risk groups on the basis of history and ultrasound scan results. The low-risk group would undergo endometrial biopsy and the higher risk would undergo immediate visualisation and biopsy of the endometrium for definitive tissue diagnosis.

We propose an algorithm (Norwich DEFAB) for predicting the risk of endometrial carcinoma on the basis of the odds of cancer from multiple logistic regression analysis for individual women presenting with postmenopausal vaginal bleeding. Norwich DEFAB provides a quantitative assessment of the risk of malignancy incorporating patient characteristics of diabetes, ultrasound scan assessment of endometrial thickness, frequency of bleeding, age, and BMI.

We propose that introduction of the Norwich DEFAB probabilistic model in clinical practice can improve the accuracy and efficiency of diagnostic work-up. For women at high risk of malignancy further diagnostic evaluation is indicated even if the initial tests were negative. Depending on prior evaluation, a combination of repeat endometrial biopsy or hysteroscopy should be pursued.

For example, a 70-year-old woman with a BMI of 35, who presents with a 2-week episode of vaginal bleeding, would have a Norwich DEFAB score of 6 (age = 1, BMI = 1, recurrent bleeding = 4) if no other risk factors are present. According to current practice, if endometrial thickness measures less than 5 mm on transvaginal ultrasound scan, no further testing would be offered to the patient; only if the patient has an ultrasound scan showing endometrial thickness greater than 5 mm, would an endometrial Pipelle biopsy be performed. However, as this patient is at increased risk of having endometrial malignancy according to the DEFAB score, we suggest that further testing, including endometrial biopsy, should be offered regardless of endometrial thickness measurement. If the biopsy does not show any abnormality, we suggest hysteroscopic evaluation of the endometrium (Chart 1).

We recommend that a Norwich DEFAB cut-off score equal to or greater than 3 should be used to consider further investigations. At this cut-off point, high sensitivity (81.9%) is achieved. Although specificity appears to be low (50.1%), this is not clinically important when considering that the primary objective is not to miss cases of malignancy. A trade-off between sensitivity and specificity is observed as the Norwich DEFAB score increases. For a Norwich DEFAB score of 5 sensitivity decreases to 67.8% and specificity increases to 74.1%.

When developing the predictive model in our study, we analysed type-I and type-II endometrial cancer cases in the same group. Although there are publications showing that women with type-II endometrial cancer have different clinical characteristics when compared with women with type-I endometrial cancer, recent evidence suggests that there is no difference in the age of diagnosis of both types of the disease. Also, obesity increases the risk of both type-I and type-II endometrial cancer (Bjorge *et al*, 2007). In addition, no difference was observed in the results of the predictive model when investigated in women with type-I cancer separately. This was not surprising as we had a small number of women with

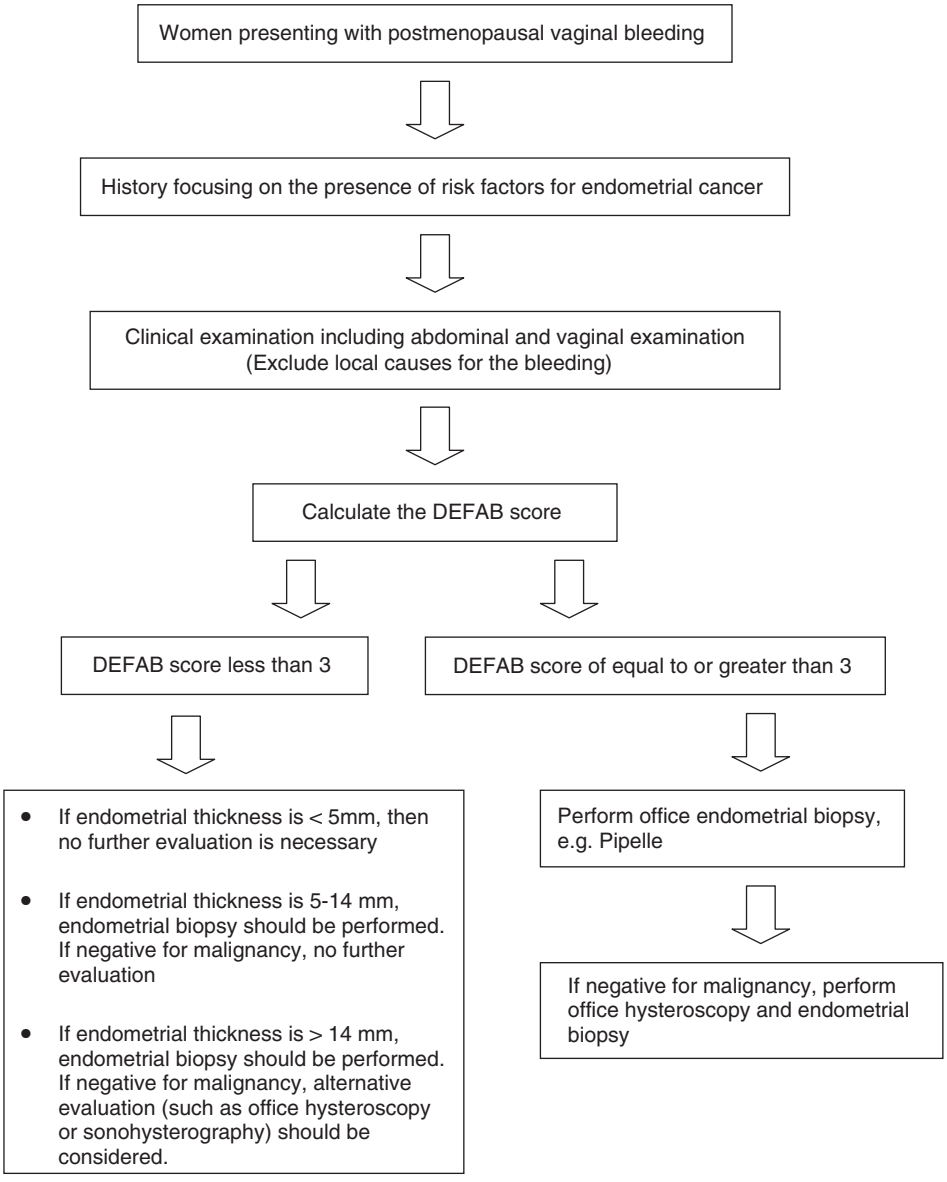

Chart 1 The proposed algorithm for management of women with postmenopausal vaginal bleeding.

type-II cancer (21 cases). Further we believe that the model should include all endometrial cancers, as it is not possible to distinguish between the two types at initial presentation of the patient, when applying the algorithm.

One of the limitations of this study is the fact that cases where endometrial thickness measurement was less that 5 mm were attributed to genital tract atrophy and no further investigation was performed. This was a pragmatic study based on the current practice in our unit where transvaginal ultrasonography is used as the initial tool to select patients who require further investigation. This practice is based on the recommendations and evidence mentioned above (ACOG Committee Opinion No 440, 2009). To evaluate the applicability of our findings in other populations, external validation of the predictive model at different cut-off points is required. External validation is also required before introduction of this model in clinical practice.

In conclusion, we have shown that incorporation of clinical information with an initial investigation tool into a risk prediction model allows assessment of the probability of the disease, which may be used to refine subsequent investigations and treatment strategies. This not only has benefit in the process of disease detection but also may result in improved efficiency of care.

It is not yet certain whether application of the Norwich DEFAB in clinical practice will have an effect on the prognosis for endometrial cancer. This should be a topic for further research.

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To whom it may concern,

I confirm that Dr Nikolaos Burbos had the following contributions to the above manuscript:

1. He conceived the idea for the study.
2. He designed and database for the data collection and collected more than 75% of the data included in the study. He also had an oversight of the data collected by his colleagues.
3. A medical statistician carried out the analysis of the data. Dr Burbos arranged study group meetings to plan the analysis, review the results, interpret and present the clinical significance of the findings.
4. He wrote the manuscript, incorporated changes from his co-authors and submitted the manuscript for publication. He responded to the comments made by the reviewers and submitted the revised version of the manuscript.

Yours sincerely,

Joaquin J. Nieto

Chapter 3. Estimating the risk of endometrial cancer in symptomatic postmenopausal women. A novel clinical prediction model based on patients' characteristics.

3.1 Introduction

In the previous chapter, I presented the development of a predictive model for women presenting with postmenopausal vaginal bleeding (PMB) using a combination of clinical characteristics and endometrial thickness measurement on ultrasonography. It is important however, to develop a similar model that can be used in the primary care setting where transvaginal ultrasonography (TVUS) is not easily available. A risk stratification approach will guide the priority for further diagnostic testing.

3.2 Literature review

Data from the literature suggest that often, women experiencing PMB may not be referred for investigation to secondary care. Using information extracted from a primary care database, McBride et al attempted to explain the variation in the patterns of referrals to secondary care for women with PMB [159]. In this cohort study, clinical and demographic records of 5492 women presenting with PMB were examined. Among them, 3374 (61.4%) women were referred to secondary care for investigation of PMB. The authors found the likelihood of referral to secondary care for PMB decreased with increasing age ($p < 0.001$) and increasing in the comorbidity score ($p < 0.001$). There was no difference in the referral pattern by social deprivation status in a multivariate analysis. The authors suggested that the variation in the referral patterns may partially reflect the clinician's uncertainty about the improvement in benefits for older or medically unfit patients [159]. In addition to clinician assessment of the likelihood and seriousness of the condition, patient-related factors such as demographic and social characteristics and patient's preferences affect the decision to refer to secondary care [160]. Psychological factors such as clinician's willingness to take risks may also play an important role in the referral pattern [160]. Variations in referral rates also exist as the

general practitioner is asked to counsel the patient and refer based on the presence of a symptom only, without considering the background risk of the condition for the particular individual.

Several predictive models have been developed to predict the risk of endometrial cancer in patients with PMB. However, most of these were derived using a combination of clinical characteristics and sonographic findings [153, 156-158], or a combination of ultrasonographic characteristics only [125, 155]. However, prompt access to high quality TVUS to characterise the endometrium is often not possible in primary care settings, thus these predictive models are not helpful to the general practitioner when assessing women with PMB.

Some predictive models using only patient clinical characteristics have been developed, the details of which were presented in the previous chapter [152, 154, 156, 158]. However, these models have significant limitations (again previously discussed), and thus may still not be helpful in the primary care setting.

3.3 What does this study add?

The selected predictors included in the development of the model presented in this study are based on patient's demographic and clinical characteristics only. This model can assist with risk stratification for women with PMB and can be used to triage patients to diagnostic tests.

3.4 What did we do well?

The predictive model that was presented in the previous chapter can be used by clinicians in secondary care, but has limited value in the primary care setting where women with PMB initially present. This is because the value of one of the predictors included in the previous model is dependent on the use of TVUS. A predictive model based on demographic and clinical characteristics only can be used in the primary care setting, if validation in this setting is successful.

One of the important changes in the current study was the involvement of the statistician at an early stage in the design of the project. This was an important lesson learned from previous experience. This approach facilitated better understanding of the research goals and more efficient preparation of the manuscript. In addition, the preparation and submission of this manuscript helped me to gain better understanding of the reviewing process for medical journals.

3.5 What could have been done differently?

It is uncertain if all women with PMB seen in primary care during the study period were referred to secondary care for investigations. If selective referrals to secondary care have taken place, the distribution of predictors and outcomes will be different between the two settings. That is likely to affect the predictive performance of the model when validated in primary care.

Estimating the Risk of Endometrial Cancer in Symptomatic Postmenopausal Women

A Novel Clinical Prediction Model Based on Patients' Characteristics

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Introduction: The aim of this study was to develop a multivariable model to predict the risk of endometrial carcinoma in postmenopausal women with vaginal bleeding using individuals' clinical characteristics.

Patients and Methods: This prospective study of consecutive postmenopausal women presenting with vaginal bleeding was conducted at a gynecological oncology center in the United Kingdom for a 46-month period. All women underwent transvaginal ultrasound scanning as the initial investigation tool to evaluate the endometrium. Women found to have an endometrial thickness 5 mm or more had endometrial sampling performed.

Results: Of a total of 3548 women presenting with vaginal bleeding during the study period, 201 (6%) women had a diagnosis of endometrial carcinoma. An investigator-led best model selection approach used to select the best predictors of cancer in the multiple logistic regression model showed that patient's age (odds ratio [OR], 1.06), body mass index (OR, 1.07), recurrent episodes of bleeding (OR, 3.64), and a history of diabetes (OR, 1.48) increased the risk of endometrial malignancy when corrected for other characteristics. The mentioned clinical variables satisfied the criteria for inclusion in our predictive model called FAD 31 (F for the frequency of bleeding episodes, A for the age of the patient, D for diabetes, and the number 31 represents the BMI cut-off value). The total score for the model varies from 0 to 8. The area under the receiver operating characteristics curve for the developed model was 0.73 (95% confidence interval, 0.70–0.77).

Discussion: We have developed a simple model based on patients' clinical characteristics in estimating the risk of endometrial cancer for postmenopausal women presenting with vaginal bleeding. The model shows reasonable discriminatory ability for women with cancer and without, with an area under the receiver operating characteristics curve of 0.73. This will allow clinicians to individualize the diagnostic pathway for women with postmenopausal vaginal bleeding.

Key Words: Predictive model, Postmenopausal bleeding, Endometrial cancer

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Endometrial carcinoma represents the most common cancer of the female genital tract and 5% of all female cancers with 7536 cases diagnosed in 2007 in the United Kingdom.[1] More than 90% of postmenopausal women diagnosed with endometrial cancer present with vaginal bleeding.[2] However, in most cases, the etiology of postmenopausal vaginal bleeding is due to benign conditions such as genital tract atrophy or endometrial polyps. There is substantial variability in the likelihood of endometrial carcinoma across postmenopausal women presenting with vaginal bleeding. The incidence of malignancy varies from 1% to 24% depending on the presence of risk factors for endometrial carcinoma and the population studied.[3–7] Thus, the clinical approach to postmenopausal bleeding requires prompt and efficient evaluation to exclude or diagnose carcinoma.[8]

Currently, in the United Kingdom, transvaginal ultrasound is commonly used as the first-step diagnostic tool in the investigation of women with postmenopausal vaginal bleeding. A thin endometrium is associated with a low risk of endometrial disease.[9] Patients with increased endometrial thickness demonstrated by ultrasound are selected for further evaluation by office-based endometrial biopsy or hysteroscopy with directed biopsy. Alternatively, office-based endometrial biopsy such as Pipelle device (Pipelle de Cornier; Laboratoire CCD, Paris, France) can be used as the initial diagnostic test to exclude endometrial cancer. However, if the office-based endometrial sampling device does not yield sufficient tissue for a robust exclusion of malignancy, the clinician is in doubt over whether to rely on the negative biopsy and reassure the patient without the need for further investigations such as hysteroscopy. In such a situation, information gained by clinical history such as age of the patient, body mass index (BMI), presence of diabetes, or hypertension can be used to individualize the work-up diagnostic strategy, based on the relative risks of these factors.

A prior knowledge of the individuals' risk of malignancy based on the presence or absence of risk factors associated with the development of endometrial cancer may lead to more efficient and cost-effective use of diagnostic tests by triaging women at high risk of endometrial cancer for histological testing or providing reassurance to women with a very low risk even without the use of ultrasound.[10]

Several predictive models incorporating clinical characteristics in estimating the risk of endometrial cancer in women presenting with postmenopausal vaginal bleeding have been developed.[11–16] The number of patients investigated in most of the studies was relatively small, and different variables were incorporated in each model, often including the results of investigations such as ultrasound or hysteroscopy. This does not allow risk estimation at the time of initial presentation to primary care because the results of the clinical investigations are not available at that time.

The aim of this study was to develop a multivariable model to predict the risk of endometrial carcinoma in women with postmenopausal vaginal bleeding using the patients' clinical characteristics without incorporating the results of clinical investigations, in particular endometrial thickness result. The ability to provide a risk assessment without the requirement of a pelvic ultrasound would enable such assessments to be performed in a primary care setting.

PATIENTS AND METHODS

This prospective cross-sectional study of consecutive postmenopausal women presenting with vaginal bleeding was conducted at a gynecological oncology center in the United Kingdom, between February 2006 and December 2009. Menopause was defined as at least 12 months of amenorrhea. Excluded from the study were premenopausal women, asymptomatic women with an incidental finding of increased endometrial thickness on imaging, asymptomatic women with abnormal endometrial cytology found on cervical smear, and women with a history of hysterectomy.

All women underwent transvaginal ultrasound scanning as the initial investigation tool to evaluate the endometrium. We used grey-scale ultrasound to measure the double-wall endometrial thickness in an anteroposterior dimension, in the sagittal plane from one basalis layer to the other. In keeping with departmental guidelines, when the endometrial thickness measured less than 5 mm, no further investigations were performed because evidence suggests a low probability of cancer below this threshold.[17,18] In 3.8% of the patients, the endometrial thickness was not identified using transvaginal ultrasound. Endometrial biopsy was performed for all these women where endometrial thickness was not clearly visualized and malignancy was diagnosed in 14.7% of cases.

Women found to have an endometrial thickness 5 mm or greater had endometrial sampling performed using an endometrial Pipelle device. Endometrial biopsy was also performed in cases where the endometrial thickness was not clearly visualized on transvaginal ultrasound. Hysteroscopic evaluation of the endometrium with biopsy under a general anesthetic was performed if Pipelle biopsy was not possible or did not yield sufficient tissue for histological diagnosis. Hysteroscopy was also performed in cases where endometrial thickness measurement by ultrasound was greater than 10 mm, in spite of benign histology on Pipelle biopsy.

Routine data regarding essential clinical information and the presence of risk factors for endometrial cancer were collected using a predesigned proforma. The following characteristics were recorded for all women: age of the patient at presentation, BMI calculated as weight (kg)/[height (m)],[2] use of hormone replacement therapy, presence of hypertension and diabetes, previous history of breast cancer, use of tamoxifen at presentation, amount of blood lost, and frequency of the episodes of vaginal bleeding. Endometrial thickness was measured by ultrasound scan, and the result of histology, when performed, was also recorded.

All the data analyzed were collected as part of the routine investigations and treatment. The patients were investigated according to established evidence-based departmental guidelines, and the individuals' data were anonymized. The ultrasonographic studies were performed by experienced examiners. There were five main investigators that performed the transvaginal scans during the study period.

For the purpose of the study, we considered all women with an endometrial thickness measurement of less than 5 mm as negative for endometrial cancer. In the same

group, we included women with benign endometrial histology including atrophy, benign polyps, endometritis, or proliferative endometrium.

Statistical Analysis

The distributions of continuous variables were not symmetric. To test for normality, the Shapiro-Wilk *W* test was used, as was the q-q plot to investigate normality graphically (results not shown). There was no evidence to suggest that data was normally distributed; hence, in the descriptive statistics for continuous variables, we report median and interquartile range. To avoid inflating the type I error rate, loss of power, residual confounding, and bias, continuous predictor variables were not categorized.[19–21] To test any differences, we used a nonparametric Wilcoxon rank sum (Mann-Whitney) test. Binomial exact methods were used to calculate 95% confidence intervals (CIs) of the proportions and to test any differences in the proportions observed. χ^2 test was used after checking the expected assumptions. An investigator-led best model selection approach was used to select the best predictors of cancer in the multiple logistic regression model as opposed to machine-led stepwise regression, which is not advisable.[22,23] Selection of predictor variables was carried out by using the likelihood ratio test after estimation of the nested models by adding and eliminating variables one at a time. The likelihood ratio test is similar to using model selection indices such as Akaike information criterion or Bayesian information criterion. All analyses were done using STATA software, version 10.1 SE (Stata Corp, College Station, TX).

RESULTS

For a 46-month period, 3548 postmenopausal women presented with vaginal bleeding were included in the study. A total of 201 (6%) women had a diagnosis of endometrial carcinoma. The remaining 3347 (94%) women were included in the noncancer group for the purposes of the analysis. The median age in the group of women diagnosed with endometrial cancer was 65 years (95% CI, 60–73 years); and in the noncancer group, 59 years (95% CI, 54–67 years). The results of univariate analysis are summarized in Table 1. The univariate analysis showed that women diagnosed with endometrial cancer were older ($P < 0.0001$) and had higher BMI ($P < 0.0001$) compared with women without cancer. Women in the endometrial cancer group were more likely to have a history of diabetes ($P < 0.0001$), hypertension

TABLE 1. Basic characteristics of individuals

	Endometrial Cancer		
Risk Factors	**Yes, n = 201 (6%)**	**No, n = 3347 (94%)**	***P***
Age (range), yr	65 (60–73)	59 (54–67)	<0.0001†
BMI (range), kg/m^2	31 (27–37)	28 (25–32)	<0.0001†
Duration of HRT, yr	14.5 (4–20)	4 (2–10)	0.033†
Tamoxifen use, yr	5 (2–8)	3 (2–5)	0.055†
Amount of bleeding*			
Spotting	49 (25%, 19%–32%)	671 (19%, 20%–22%)	
Light	111 (57%, 49%–64%)	1936 (59%, 57%–60%)	0.270‡
Heavy	36 (18%, 13%–25%)	687 (21%, 19%–22%)	
Frequency of bleeding*			
Single episode	45 (23%, 17%–29%)	1738 (52%, 50%–54%)	<0.0001‡
Recurrent	155 (78%, 71%–83%)	1597 (48%, 46%–50%)	
Diabetes			
No	173 (86%, 80%–91%)	3163 (94%, 94%–95%)	<0.0001‡
Yes	28 (14%, 9%–20%)	184 (6%, 5%–6%)	
Hypertension			
No	122 (62%, 54%–70%)	2498 (75%, 73%–76%)	<0.0001‡
Yes	79 (39%, 33%–46%)	849 (25%, 24%–27%)	
Breast cancer			
No	178 (89%, 83%–93%)	3132 (94%, 93%–94%)	0.006‡
Yes	23 (11%, 7%–17%)	215 (6%, 6%–7%)	

Values are median (interquartile range [IRQ]), number (percent, 95% CI of percent).
*Percentages worked on less numbers from the overall due to missing values.
†Two-sample Wilcoxon rank sum test (Mann-Whitney test).
‡χ^2 test.
HRT, Hormone replacement therapy.

TABLE 2. Adjusted predictors of endometrial malignancy (OR) from the best model that fits the data well

Predictors of Malignancy	OR	95% CI	*P*
Age, yr	1.06	1.04–1.07	<0.0001
BMI, kg/m^2	1.07	1.05–1.09	<0.0001
Frequency of bleeding			
Single episode		1	
Recurrent episodes	3.64	2.55–5.15	<0.0001
Diabetes			
No		1	
Yes	1.48	1.06–2.37	0.023

($P < 0.0001$), or previously have a diagnosis of breast cancer ($P = 0.006$).

We also investigated in our cohort if the individual's pattern of vaginal bleeding had any effect on the predictive value with regard to the diagnosis of endometrial malignancy. The amount of vaginal bleeding was recorded as spotting, less than a period, or light period. There was no evidence ($P = 0.270$) that the amount of bleeding alters the likelihood of an individual with a diagnosis of endometrial cancer. However, there was strong evidence ($P < 0.0001$) that recurrent episodes of vaginal bleeding are associated with an increased risk of endometrial cancer.

Development of the Clinical Prediction Model

An investigator-led best model selection approach in the multiple logistic regression to determine the best predictors of endometrial cancer showed that patient's age (odds ratio [OR], 1.06; 95% CI, 1.04–1.07), BMI (OR, 1.07; 95% CI, 1.05–1.09), recurrent episodes of bleeding (OR, 3.64; 95% CI, 2.55–5.15), and a history of diabetes (OR, 1.48; 95% CI, 1.06–2.37) increased the risk of endometrial cancer when corrected for other characteristics (Table 2). The mentioned clinical variables satisfied the criteria for inclusion in our predictive model called FAD 31: F for the frequency of bleeding episodes, A for the age of the patient, D for diabetes, and the number 31 represents the BMI cut-off value used. The total FAD 31 score is calculated by adding the score for each clinical characteristic included: recurrent episodes of bleeding score 4; age 65 years or older scores 1; a history of diabetes scores 2; and a BMI 31 kg/m^2 or greater scores 1. When a criterion is absent, the score is equal to 0. The total score for the FAD 31 varies from 0 to 8. The score for each variable was derived with respect to the predictive odds ratio of each variable in the adjusted logistic model after arriving at the best model using the likelihood ratio test.

TABLE 4. Sensitivity, specificity, PPV, NPV of FAD 31

	FAD 31 Score ≥4	
	Estimate	95% CI
Sensitivity	80.1%	73.9%–85.4%
Specificity	51.0%	49.3%–52.7%
ROC area	0.656	0.627–0.685
d-OR	4.19	2.95–5.96
PPV	8.94%	7.67%–10.4%
NPV	97.7%	96.9%–98.4%

NPV, Negative predictive values; PPV, positive predictive value.

Table 3 shows the sensitivity, specificity, and ability for correct classification of the FAD 31 at different cut-off values. A trade-off between the sensitivity and specificity is observed with increasing FAD 31 values. A FAD 31 score of 4 or higher shows a more balanced trade-off between sensitivity and specificity as presented in Table 4. An important result at this threshold is the high negative predictive value of 97.7% (95% CI, 96.9–98.4). Figure 1 shows the area under the receiver operating characteristics curve (ROC curve) that represents the discriminatory ability of the model

TABLE 3. Overall sensitivity, specificity, correct classification for each FAD 31 cut-off point

Cut-Off Point	Sensitivity, %	Specificity, %	Correctly Classified, %	LR(+)	LR(−)	d-OR
(≥0)	100.00	0.00	5.66	1.000		—
(≥1)	95.50	23.15	27.24	1.243	0.194	6.41
(≥2)	86.50	44.77	47.13	1.566	0.302	5.19
(≥3)	81.00	49.78	51.54	1.613	0.382	4.22
(≥4)	80.00	51.21	52.84	1.640	0.391	4.19
(≥5)	65.00	72.65	72.22	2.377	0.482	4.93
(≥6)	28.50	92.74	89.11	3.928	0.771	5.09
(≥7)	10.00	97.48	92.53	3.970	0.923	4.30
(≥8)	6.50	99.13	93.89	7.475	0.943	7.93
(>8)	0	100.0	94.34	—	1.000	

d-OR, Diagnostic odds ratio = LR(+)/LR(−); LR(−), likelihood ratio negative; LR(+), likelihood ratio positive.

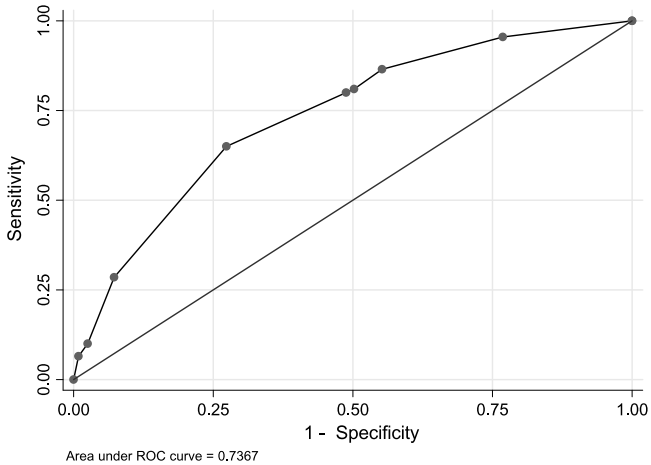

FIGURE 1. Receiver operating characteristic curve for FAD 31.

was 0.73 (95% CI, 0.70–0.77). This demonstrates a reasonable capacity to discriminate between women with endometrial cancer and those without the disease.

DISCUSSION

Currently, there are no criteria in use for stratifying patients presenting with postmenopausal vaginal bleeding into well-defined risk groups with respect to developing endometrial cancer. In this study, we evaluated the incorporation of already known clinical risk factors into a statistical predictive model for endometrial cancer in symptomatic postmenopausal women. An important advantage of our predictive model is that it can be applied at an early stage in the patient's referral pathway, for example, in the primary care setting where patients often initially present with postmenopausal vaginal bleeding. The high negative predictive value, which is observed at FAD 31 cut-off scores of less than 4, can be used to prioritize the referrals to secondary care allowing women at low risk of endometrial malignancy to be referred on a less urgent basis. This may reduce the strain on the currently available resources in the secondary care. In addition, the high negative predictive value at different cut-off scores can facilitate the clinician's decisions regarding the need for further investigation in cases of inadequate specimen obtained from office-based endometrial sampling devices, for example, a patient at low risk of cancer that underwent outpatient sampling of the endometrium may not require hysteroscopic evaluation even in cases where the tissue specimen is insufficient to provide a reliable diagnosis. In a similar way, women with a high probability of endometrial cancer (FAD 31 scores of 4 or higher) should undergo endometrial biopsy for the initial evaluation of the endometrium.[10] With the increasing use of hysteroscopy in outpatient settings, women at high risk of endometrial malignancy should be triaged to specialist clinics that offer visualization and tissue biopsy of the endometrium (Fig. 2).

Our predictive model showed similar discriminatory ability with the model developed by Opmeer et al[16] (area under ROC curve, 0.73 and 0.76). Similarly, both studies have evaluated the extent to which every factor contributes to the risk of endometrial cancer. However, in the study by Opmeer et al,[16] additional risk factors for endometrial cancer included in the predictive model were use of anticoagulants and nulliparity. We did not include parity of women in our predictive model because nulliparity per se does not appear to increase the risk of endometrial cancer, but there may be an association in subfertile women with a high frequency of anovulatory cycles.[24] In addition, in this study, a BMI greater than 26 kg/m^2 was strongly associated with increased risk of malignancy. This appears to be very low when compared with women with endometrial cancer in our study population (median BMI = 31; 95% CI, 27–37). The cut-off age of women in the model developed by Opmeer et al[16] was younger than in our study (55 years vs 65 years, respectively).

Other case-control studies that proposed scoring systems for prediction of endometrial cancer have shown similar results.[12,25] The risk factors that were incorporated into the models derived from the mentioned studies varied. Also, the studies included in their analysis premenopausal women with endometrial cancer. The retrospective design of these studies leads to significant bias because only a small proportion of the controls were studied, leading to underestimation of the prevalence of the disease. However, the multivariate analyses from these studies have incorporated increasing age, obesity, and diabetes as significant risk factors in their predictive models. Other authors have included the time elapsed since the menopause to improve the predictive ability of the model.[15] In our population, we did not collect the data regarding the duration of the menopause because there is significant variation in the ability of patients to recall the exact time of their last menstrual period.

There are a few limitations of our study. This is a single institution study, and the results may not be easy to generalize due to biases arising from the characteristics of the population studied. Nevertheless, this is the largest prospective study to date in the evaluation of symptomatic postmenopausal women, which helps to minimize the bias. Although the study was performed in a secondary care center, the incidence of endometrial cancer in this cohort of women represents a true reflection of that encountered in the primary care setting. This is a consequence of the current national recommendations, according to which all women presenting with postmenopausal vaginal bleeding should be referred to secondary care in order to exclude malignancy.

In our unit, we did not perform endometrial biopsy for women with endometrial thickness measurement less than 5 mm, although we appreciate that ultrasound cannot entirely exclude malignancy below this threshold.[17,18] We accept that our study design may have underestimated the presence of cancer in cases where endometrial thickness measured less than 5 mm. However, this is a pragmatic study, and the practice is based on the recommendations suggesting that women with postmenopausal uterine bleeding may be assessed initially with either endometrial biopsy or transvaginal ultrasound; this initial evaluation does not require performance of both tests.[2,8] In addition, we searched our database and found that among patients with endometrial cancer during the study period, only one was previously

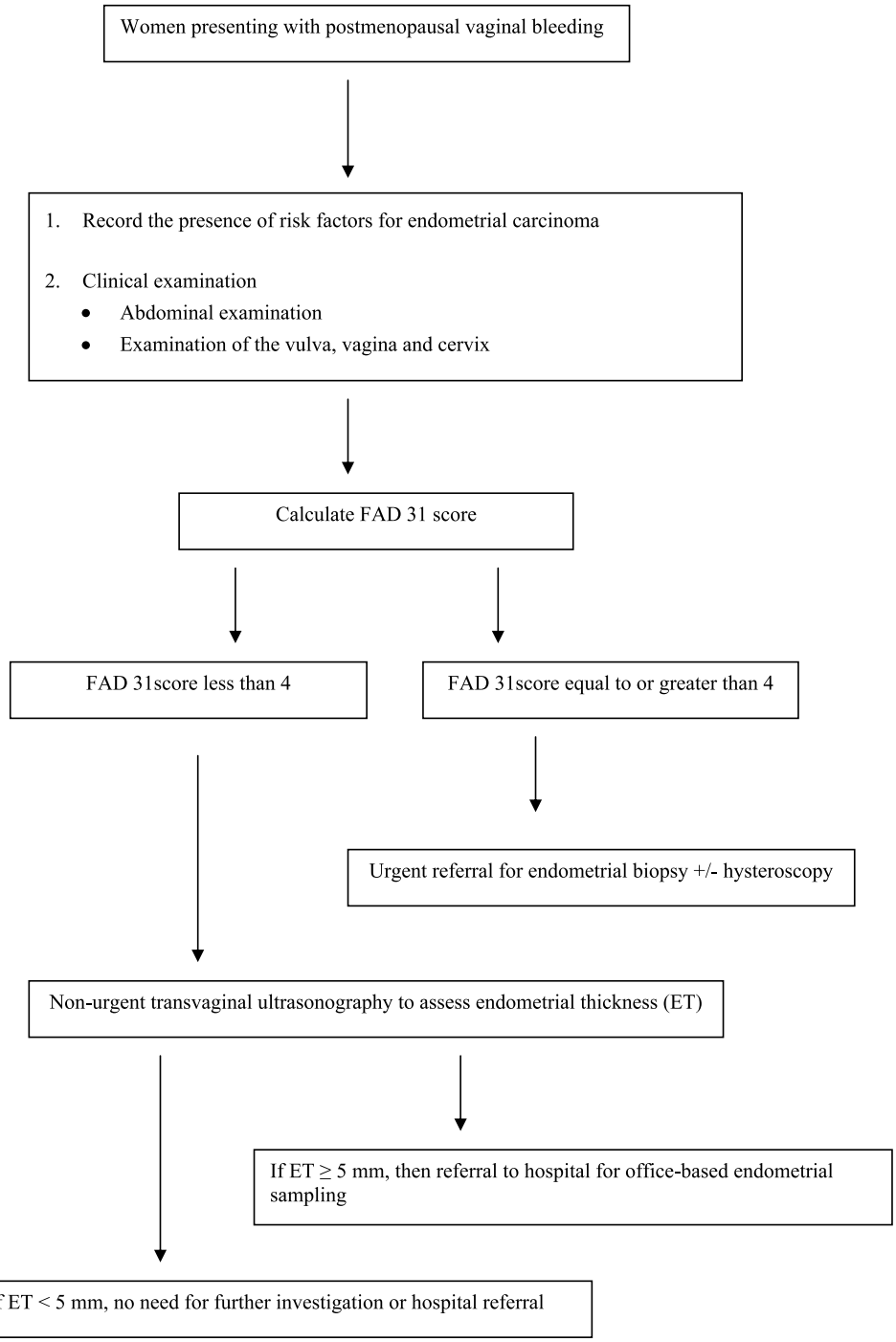

FIGURE 2. Risk assessment pathway for the management of women with postmenopausal bleeding.

investigated and found to have an endometrial thickness measuring less than 5 mm on transvaginal ultrasound. During this period, 1762 women were found to have an endometrial thickness measuring less than 5 mm on ultrasound. This results in a 0.0005 incidence of cancer among this group of women in our study population. In addition, the region where this study took place is characterized by a stable population; hence, it is unlikely that cases of cancer may have been investigated elsewhere.

In conclusion, we have developed a simple model based on patients' clinical characteristics in estimating the risk of endometrial cancer for postmenopausal women presenting with vaginal bleeding. Introduction of the predictive model in clinical practice will help to streamline the referral

and investigation patterns for women with postmenopausal vaginal bleeding. The model shows reasonable discriminatory ability for women with cancer and without, with an area under ROC curve of 0.73. Our aim is to computerize the predictive model such that when the data from the 4 clinical parameters (age, BMI, diabetes, and frequency of bleeding episodes) are entered, the risk of endometrial cancer is calculated automatically. This will allow clinicians to individualize the diagnostic pathway for women with postmenopausal vaginal bleeding. Further research is required to externally validate the predictive model developed in order to assess its clinical applicability in different populations.

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To whom it may concern,

I confirm that Dr Nikolaos Burbos had the following contributions to the above manuscript:

1. He conceived the idea for the study.
2. He designed and database for the data collection and collected more than 75% of the data included in the study. He also had an oversight of the data collected by his colleagues.
3. A medical statistician carried out the analysis of the data. Dr Burbos arranged study group meetings to plan the analysis, review the results, interpret and present the clinical significance of the findings.
4. He wrote the manuscript, incorporated changes from his co-authors and submitted the manuscript for publication. He responded to the comments made by the reviewers and submitted the revised version of the manuscript.

Yours sincerely,

Joaquin J. Nieto

Chapter 4. Comparing the performance of two clinical models in estimating the risk of endometrial cancer in symptomatic postmenopausal women.

4.1 Introduction

In this chapter, I describe the internal validation of the predictive models that were presented in the previous chapters. This is a necessary step in the development of predictive models.

4.2 Literature review

Predictive models are algorithms developed using patient-level data to estimate the probability of the individual being diagnosed or developing a particular condition [161]. A diagnostic predictive model predicts the probability of a condition at the time of the individual's presentation, while a prognostic model is used to provide an estimate of the probability of the condition developing in the future [162]. When a model is developed it is usual to utilise multiple predictors derived from patient clinical characteristics, laboratory data or imaging results.

Logistic regression is used to predict a binary endpoint when developing diagnostic predictive models [163]. All relevant clinical variables should be included, even if there is no statistical significance observed in univariate analysis [164]. Confounding by other variables may affect the results on univariate analysis and lead to important predictors being omitted from the predictive model [165].

Two main characteristics that determine the performance of predictive models in medicine include discrimination and calibration [161]. Discrimination is the ability of the model to accurately predict those with the condition and those without it [161]. Several measures such as the area under the receiver operating characteristics curve, box plots or Lorenz curves can be used to report discrimination [166, 167].

Calibration refers to the agreement between observed outcomes and predictions [168]. For a well-calibrated model, the predicted probabilities should match closely the observed frequencies. It is recommended that calibration should always be evaluated and reported in the development of predictive models [164].

Prior to implementing a model in clinical practice, the validity of the predictions needs to be tested. Predictive models are designed to optimally fit the data in the development sample and the results may not be valid in new samples [163]. Hence, validation is an important part of predictive model development [164]. Internal validation assesses the validity of the model for the setting the data originated from, while external validation is performed using a dataset different to the one used to develop the predictive model [164]. Several techniques such as apparent validation, split-sample validation, cross-validation and bootstrap validation, are available for assessing internal validity [164]. Internal validation, although helpful, does not provide information about the models performance elsewhere [169].

4.3 What does this study add?

The results of this study confirm good discriminatory ability of two clinical models in predicting the probability of a patient being diagnosed with endometrial cancer. The study also showed there was no significant difference in the discrimination ability of both models.

4.4 What went well?

Internal validation is an essential part of predictive model development and was considered by the study team. Preparation of this manuscript gave me the opportunity to improve my knowledge in the statistical methods used in medical research. I attended on-line research seminars, read medical statistics books and organised study groups with my colleagues. This was also an opportunity for me to expand my knowledge and skills in the use of statistical software. This knowledge has been particularly useful, not only for working in new research projects but also to better understand published data and critically appraise the literature.

4.5 What could have been done differently?

Calibration of the predictive models was not presented in the manuscript. We should have considered publishing a calibration plot for the models.

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Comparing the performance of two clinical models in estimating the risk of endometrial cancer in symptomatic postmenopausal women

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ABSTRACT

Objective: The aim of this study was to internally evaluate the accuracy measures of the two newly developed predictive models, called DEFAB and DFAB, used to estimate the risk of endometrial cancer in postmenopausal women presenting with vaginal bleeding.
Study design: Prospective study including postmenopausal women presenting with vaginal bleeding.
Results: Over a 46-month-period, 3795 postmenopausal women presented with vaginal bleeding and were included in the study. A total of 221 (6%) women were diagnosed with endometrial carcinoma. The DEFAB predictive model incorporates known risk factors such as presence of Diabetes, Endometrial thickness measurement on transvaginal ultrasonography, Frequency of bleeding, Age, and Body mass index. The DFAB model is based on the above clinical characteristics excluding the ultrasonography result. For the recommended cut-off values, there was no evidence (p-value = 0.221) of a difference in the diagnostic ability with respect to sensitivity, specificity, area under receiver operating curve, positive predictive value and negative predictive value. There was strong evidence (p-value < 0.0001) to suggest that the diagnostic ability of DEFAB and DFAB agree as evidenced by the excellent Kappa statistic 0.950 (95% CI 0.940–0.960). We found strong evidence (p-value < 0.0001) that the variables incorporated in both predictive models simultaneously correctly classify an individual to either having cancer or not having cancer with respect to logistic discriminant analysis.
Conclusion: We recommend that these two predictive models can be used interchangeably.

1. Introduction

Postmenopausal vaginal bleeding is the presenting symptom in over 90% of women diagnosed with endometrial cancer [1]. The aim of the diagnostic work up in these women is to rule out malignancy. However, only 5–10% (range 1–24%) of women presenting with postmenopausal vaginal bleeding will be diagnosed with endometrial malignancy [2–5]. Thus, the predictive value of symptoms for endometrial cancer is relatively low and a large number of healthy women need to undergo investigations such as transvaginal ultrasonography and/or endometrial biopsy.

The objective of clinical prediction rules is to reduce the uncertainty inherent in medical practice by defining how to use clinical findings to make predictions. Clinical prediction rules are derived from systematic clinical observations. They can help physicians identify patients who require diagnostic tests, treatment, or hospitalization [6].

Several risk factors such as obesity, tamoxifen use, increasing age, hypertension, diabetes and unopposed use of exogenous oestrogens are strongly associated with increased risk of type I endometrial cancer [7–11]. We developed two predictive models for estimating the risk of endometrial cancer in postmenopausal women presenting with vaginal bleeding [12,13]. In the first model [12], through a process of an investigator led best model selection approach in the multiple logistic regression, we identified the following variables as best predictors of endometrial cancer: history of diabetes, endometrial thickness, frequency of bleeding episodes, age, and body mass index (BMI). We called the clinical prediction tool DEFAB representing Diabetes, Endometrial thickness, Frequency of bleeding, Age and BMI.

The DEFAB tool is useful in cases where ultrasonography is available and endometrial thickness can be measured as this variable is incorporated in the predictive model. However, the majority of the patients with postmenopausal vaginal bleeding are initially evaluated by their general practitioner and decision about

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their referral pathway often are made without having the knowledge of endometrial thickness measurement. In such a situation, information gained by clinical history such as age of the patient, body mass index, presence of diabetes or hypertension can be used to individualise the referral pathways and the work-up diagnostic strategy, based on the relative risks of these factors. We developed a clinical prediction tool which we called FAD 31 where F stands for frequency of bleeding episodes, A for age, D for diabetes and number 31 representing BMI cut off value used [13].

The aim of this study was to internally evaluate the accuracy measures of the two newly developed predictive models used to estimate the risk of endometrial cancer in postmenopausal women presenting with vaginal bleeding and to compare how well these two predictive models agree. We also evaluated the ability of the predictor variables to classify an individual to either having endometrial cancer or not.

2. Methods

The data were collected from a prospective cohort study of consecutive postmenopausal women presenting with vaginal bleeding conducted at a gynaecological oncology centre in the United Kingdom, between February 2006 and December 2009. Menopause was defined as at least 12 months of amenorrhoea. Excluded from the study were premenopausal women, asymptomatic women with an incidental finding of increased endometrial thickness on imaging, asymptomatic women with abnormal endometrial cytology found on cervical smear and women with a history of hysterectomy.

All women underwent transvaginal ultrasound scanning as the initial investigation tool to evaluate the endometrium. The double wall endometrial thickness was measured in an anteroposterior dimension from one basalis layer to the other. In keeping with departmental guidelines, when the endometrial thickness measured less than 5 mm no further investigations were performed as evidence suggests a low probability of cancer below this threshold [14,15].

Women found to have an endometrial thickness equal to or greater than 5 mm had endometrial sampling performed using an endometrial Pipelle® device. Endometrial biopsy was also performed in cases where the endometrial thickness was not clearly visualised on transvaginal ultrasonography. Hysteroscopic evaluation of the endometrium with biopsy under a general anaesthetic was performed if Pipelle® biopsy was not possible or did not yield sufficient tissue for histological diagnosis.

In the clinic routine data regarding essential clinical information and the presence of risk factors for endometrial cancer are collected using a pre-designed proforma. The following characteristics were recorded for all women: age of the patient at presentation, body mass index (BMI) calculated as weight (kg)/[height (m)] ^{2}, use of hormone replacement therapy, presence of hypertension and diabetes, previous history of breast cancer, use of Tamoxifen at presentation, amount and frequency of the episodes of vaginal bleeding. Endometrial thickness measured on ultrasound scan and the result of histology when performed were also recorded. The amount of bleeding was characterised as spotting, light (=less than a period) and heavy (=like a period or worse). Any event lasting less than 7 days was defined as a single bleeding episode. Recurrent episodes were defined as any bleeding episode lasting 7 or more days or two or more separate bleeding events within the last 12 months [12].

All the data analysed were collected as part of the routine investigations and treatment. The patients were investigated according to established evidence based departmental guidelines.

For the purpose of the study, we considered all women with an endometrial thickness measurement of less than 5 mm as negative for endometrial cancer. In the same group we included women with benign endometrial histology including atrophy, benign polyps, endometritis or proliferative endometrium.

2.1. Statistical analysis

There was no evidence to suggest that continuous variables were normally distributed as observed by graphical exploration of histograms being not symmetric or values not following the reference line on the q–q plot (results not shown). Further, the Shapiro–Wilk *W* test for normality was carried out. Hence in the descriptive statistics for continuous variables, we report median and inter quartile range. To avoid inflating the type I error rate, loss of power, residual confounding and bias, continuous predictor variables were not categorised [16–18]. To test any differences we used a non-parametric Wilcoxon rank sum (Mann–Whitney) test. Binomial exact methods were used to calculate 95% confidence intervals of the proportions and to test any differences in the proportions observed. Chi-squared test was used after checking the expected assumptions.

Discriminant analysis uses a number of variables to classify an individual/item into known groups for example cancer or not cancer [19–21]. To evaluate the ability of the predictor variables to classify an individual to either having cancer or not having cancer, we used the logistic discriminant analysis [22]. Logistic discriminant analysis does not assume that the effect of the best predictor variables found in our previous publications to discriminate between the two groups (in our case cancer or not cancer) will have the distribution within groups to follow a normal distribution but assume that the likelihood ratios of the groups have an exponential form [22].

We compared the specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) of the two predictive models using Binomial exact methods by testing the null hypothesis of no difference between the corresponding values of the two predictor models. We used Kappa statistics to test how well the two predictive models agree. To compare the ROC curves, we used the method described by DeLong et al. [23]. All analyses were done using STATA software, version 11.1 SE (stata Corporation, Texas, USA). PM (medical statistician) performed the statistical analyses.

3. Results

Over a 46-month-period, 3795 postmenopausal women presented with vaginal bleeding and were included in the study. A total of 221 (6%) women were diagnosed with endometrial carcinoma. The remaining 3574 (94%) women were included in the non-cancer group for the purposes of the analysis. The median age in the group of women diagnosed with endometrial cancer was 65 years (interquartile range, 60–73 years) and in the non-cancer group was 59 years (interquartile range, 54–67 years). The characteristics of individuals in the study and the results of univariate analysis are summarised in Table 1. The univariate analysis showed that women diagnosed with endometrial cancer were older ($p < 0.0001$) and had higher body mass index ($p < 0.0001$) compared with women without cancer. Women in the endometrial cancer group were more likely to have a history of diabetes ($p < 0.0001$), hypertension ($p < 0.0001$) or previously being diagnosed with breast cancer ($p = 0.001$).

We also investigated in our cohort if the individual's pattern of vaginal bleeding had any effect on the predictive value with regard to the diagnosis of endometrial malignancy. The amount of vaginal bleeding was recorded as spotting, less than a period or light period. There was no evidence ($p = 0.303$) that the amount of bleeding alters the likelihood of an individual being diagnosed

Table 1
Basic characteristic of individuals in the study.

Clinical characteristics	Cancer		*p*-Value
	Yes, *n* = 221 (6%)	No, *n* = 3574 (94%)	
Age (year)	65 (60–73)	59 (54–67)	<0.0001[a]
BMI (kg/m^2)	31 (27–37)	28 (25–32)	<0.0001[a]
Duration of HRT (years)	14.5 (4–20)	4 (2–10)	0.034[a]
Tamoxifen use (years)	5 (2.5–7.5)	3 (2–5)	0.025[a]
Bleeding[c]			
Spotting	51 (24%, 18–30%)	697 (20%, 18–21%)	
Light	127 (59%, 52–65%)	2100 (60%, 58–61%)	0.303[b]
Heavy	38 (18%, 13–23%)	724 (21%, 19–22%)	
Frequency of bleeding[c]			
Single episode	45 (20%, 15–26%)	1839 (52%, 50–53%)	<0.0001[b]
Recurrent	175 (60%, 74–85%)	1723 (48%, 47–50%)	
Diabetes			
No	192 (87%, 82–91%)	3379 (95%, 94–95%)	<0.0001[b]
Yes	29 (13%, 9–18%)	195 (5%, 5–6%)	
Hypertension			
No	132 (60%, 53–66%)	2665 (75%, 73–76%)	<0.0001[b]
Yes	89 (40%, 34–47%)	909 (25%, 24–27%)	
Breast cancer			
No	194 (88%, 83–92%)	3344 (94%, 93–94%)	0.001[b]
Yes	27 (12%, 8–17%)	230 (6%, 6–7%)	
Endometrial thickness (mm)	14.0 (11.0–20.2)	4.5 (3.0–7.8)	<0.0001[a]

Values are median (inter-quartile range), number (percent, 95% CI of percent).
HRT (hormone replacement therapy).
[a] Two-sample Wilcoxon rank sum test (Mann–Whitney test).
[b] Chi-squared test.
[c] Percentages worked on less numbers from the overall due to missing values.

Table 2
Sensitivity, specificity, PPV, NPV, of DEFAB and DFAB.

Diagnostics	DEFAB ≥ 3	DFAB ≥ 4	*p*-Value
	Estimate (95% CI)	Estimate (95% CI)	
Sensitivity	85.9% (80.6%, 90.2%)	81.8% (76.1%, 86.7%)	0.758[¥]
Specificity	48.4% (46.7%, 50.1%)	50.8% (49.1%, 52.4%)	0.764[¥]
ROC area	0.672 (0.647, 0.696)	0.663 (0.636, 0.690)	0.211[a]
d-OR	5.72 (3.90, 8.39)	4.64 (3.28, 6.57)	
PPV	9.32% (8.09%, 10.7%)	9.31% (8.05%, 10.7%)	1.000[¥]
NPV	98.2% (97.5%, 98.8%)	97.8% (97.1%, 98.5%)	0.943[¥]

PPV = positive predictive value, NPV = negative predictive values, ROC = receiver operating characteristic area. d-OR = diagnostic odds ratio = LR(+)/LR(−).
LR(+) = likelihood ratio (+ve) = Pr(+ve|+ve)/Pr(+ve|−ve), LR(−) = likelihood ratio (−ve) = Pr(−ve|+ve)/Pr(−ve|−ve).
DEFAB = diabetes (if yes, scores 2, 0 otherwise), endometrial thickness ≥ 14 mm, score 1, 0 otherwise), frequency of bleeding (recurrent scores 4, 0 otherwise, age (≥64, scores 1, 0 otherwise) BMI (≥31 scores 1, 0 otherwise).
DFAB = diabetes (if yes, scores 2, 0 otherwise), frequency of bleeding (recurrent scores 4, 0 otherwise, Age (≥64, scores 1, 0 otherwise) BMI (≥31 scores 1, 0 otherwise).
[a] Chi-squared test.
[¥] *p*-Values obtained using binomial exact methods.

with endometrial cancer. However, there was strong evidence ($p < 0.0001$) that recurrent episodes of vaginal bleeding are associated with an increased risk of endometrial cancer.

3.1. Development of the two clinical prediction models

The two prediction models under comparison here have already been published in two separate papers [12,13]. In DEFAB criteria, with respect to the odds of predicting cancer for each variable, the presence of diabetes in a patient scores 2; endometrial thickness ≥ 14 mm scores 1; frequency of recurrent bleeding episodes scores 4; age ≥ 64 years scores 1 and BMI ≥ 31 kg m^{-2} scores 1. If the criterion is absent, then the score is 0. The calculated Norwich DEFAB score can vary from a value of 0–9. The score range for the FAD 31 tool varies from 0 to 8. Actually, for easy comparisons with the DEFAB tool, in this paper we will call this tool DFAB to emphasise the fact that the only difference between DEFAB tool and DFAB tool is that DEFAB includes endometrial thickness measurement whereas DFAB does not include endometrial thickness.

Our recommended cut-off points in DEFAB is a score ≥ 3 whereas for DFAB is a score ≥ 4. Interest is to compare these two clinical prediction tools so as to see whether one tool can be used in place of the other. Table 2 shows the results of comparing the DEFAB and DFAB tool with respect to sensitivity, specificity, ROC area, positive predictive value and negative predictive value. We can see that on each measure, we have no evidence (*p*-values > 0.05) to suggest that there was any difference between these two models used. Furthermore, the diagnostic odds ratios of DEFAB and DFAB is very high (d-OR = 5.72 and 4.64 respectively). In other words, the odds of being correctly classified as having cancer is 5.72 times greater if you have a score ≥ 3 in the DEFAB tool and the odds of being correctly classified as having cancer is 4.64 times greater if you have a score ≥ 4 with the DFAB tool.

Table 3
Overall sensitivity, specificity, correct classification for each DEFAB cut-off point.

Cut-point	Sensitivity	Specificity	Correctly classified	LR(+)	LR(−)	d-OR
(≥0)	100.00%	0.00%	5.82%	1.000	–	–
(≥1)	97.27%	21.50%	25.91%	1.239	0.127	9.76
(≥2)	92.27%	42.08%	45.00%	1.593	0.184	8.66
(≥3)	85.91%	48.40%	50.58%	1.665	0.291	5.72
(≥4)	82.73%	50.62%	52.49%	1.675	0.341	4.91
(≥5)	74.09%	70.21%	70.44%	2.487	0.369	6.74
(≥6)	49.09%	90.37%	87.97%	5.098	0.563	9.06
(≥7)	19.09%	96.04%	91.57%	4.823	0.842	5.73
(≥8)	7.73%	98.79%	93.50%	6.401	0.934	6.85
(≥9)	3.64%	99.80%	94.21%	18.504	0.966	19.16
(>9)	0.00%	100.00%	94.18%	–	1.000	–

ROC area = 0.784, 95% CI (0.753–0.814), p-value < 0.0001.
LR(+) = likelihood ratio (+ve) = Pr(+ve|+ve)/Pr(+ve|−ve), LR(−) = likelihood ratio (−ve) = Pr(−ve|+ve)/Pr(−ve|-ve).
d-OR = diagnostic odds ratio = LR(+)/LR(−).

Table 4
Overall sensitivity, specificity, correct classification for each DFAB cut-off point.

Cut-point	Sensitivity	Specificity	Correctly classified	LR(+)	LR(−)	d-OR
(≥0)	100.00%	0.00%	5.82%	1.000	–	–
(≥1)	95.91%	22.94%	27.18%	1.245	0.178	6.99
(≥2)	87.73%	44.55%	47.07%	1.582	0.276	5.73
(≥3)	82.73%	49.44%	51.37%	1.636	0.349	4.69
(≥4)	81.82%	50.79%	52.59%	1.663	0.358	4.65
(≥5)	66.36%	72.09%	71.76%	2.378	0.467	5.09
(≥6)	27.27%	92.50%	88.71%	3.638	0.786	4.63
(≥7)	9.55%	97.39%	92.28%	3.656	0.929	3.94
(≥8)	6.36%	99.05%	93.65%	6.667	0.945	7.06
(>8)	0.00%	100.00%	94.18%	–	1.000	–

ROC area = 0.740, 95% CI (0.709–0.771).
LR(+) = likelihood ratio (+ve) = Pr(+ve|+ve)/Pr(+ve|−ve), LR(−) = likelihood ratio (−ve) = Pr(−ve|+ve)/Pr(−ve|−ve).
d-OR = diagnostic odds ratio = LR(+)/LR(−).

Tables 3 and 4 show the sensitivity, specificity, ability for correct classification, likelihood ratios, and diagnostic odds ratios at different cut-off values of DEFAB and DFAB prediction tool respectively. We can see that in both tools, the recommended cut-off seems to strike a more balanced trade-off between sensitivity and sensitivity. Figs. 1 and 2 show the area under the receiver operating characteristic curve (ROC area) for the recommended cut-off point and for the overall possible cut-off for each tool. It is reassuring to note that for the recommended cut-off values, there is no evidence (p-value = 0.221) of a difference in the diagnostic ability. In addition, we have strong evidence (p-value < 0.0001) to suggest that the diagnostic ability of DEFAB and DFAB agree as evidenced by the excellent Kappa statistic 0.950 (95% CI 0.940–0.960). This indicates that using either prediction model is unlikely to result in disagreement. Hence, the two prediction models can be used interchangeably.

Using a multivariate approach [19–22], we further analysed the ability of the variables: diabetes, endometrial thickness, frequency of bleeding, age and BMI to classify an individual as either having cancer or not having cancer. We have strong evidence (p-value < 0.0001) as shown in Table 5 that these variables simultaneously correctly classifies an individual to either having cancer or not having cancer with respect to logistic discriminant analysis [22].

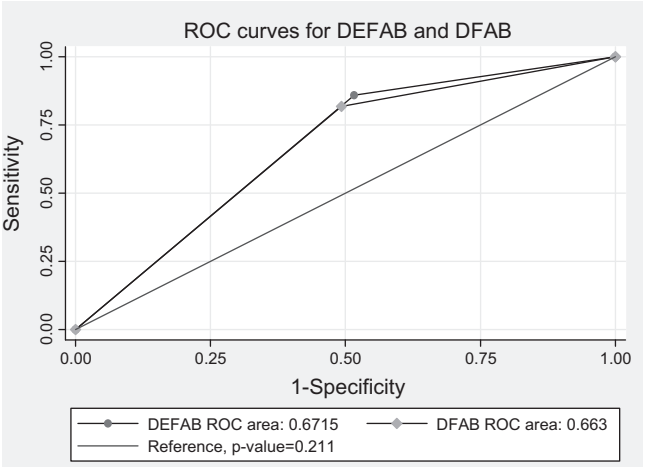

Fig. 1. ROC curve for DEFAB and DFAB cut-off values.

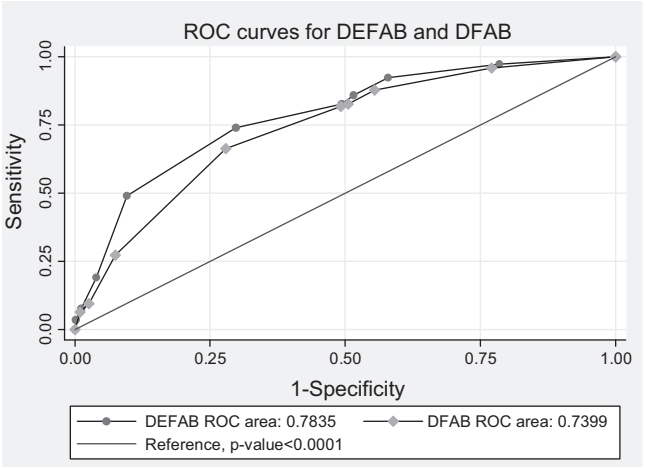

Fig. 2. ROC curve for DEFAB and DFAB.

Table 5
Logistic discriminant analysis of diabetes, endometrial thickness, frequency of bleeding, age and body mass index.

True cancer	Classification by logistic discriminant analysis		*p*-value
	No: Number (%)*	Yes: Number (%)*	
No	2761 (80%)	698 (20%)	<0.0001
Yes	35 (17%)	166 (83%)	<0.0001
Prior probability	0.50	0.50	

* Percentages worked on less numbers from the overall due to missing values on some variables.

4. Discussion

The objective of the predictive rules developed is to predict the risk of endometrial cancer in postmenopausal women presenting with vaginal bleeding. The variables incorporated in these two predictive models include known clinical characteristics that have been previously shown to be associated with increased risk of endometrial malignancy. This is of particular clinical importance as endometrial cancer is the most frequent malignancy of the female genital tract [24]. The variables included in these rules are clinically sensible and easy to apply. In addition, both rules are easy to calculate and to apply in clinical practice.

It has been suggested that the prediction rule is more likely to be used if it suggest a course of action rather than the probability of disease [25]. The DFAB score makes use of clinical characteristics in classifying patients at high and low risk of endometrial cancer. Women at high risk of malignancy can be referred urgently to specialist clinics that have the facilities for tissue biopsy and hysteroscopic evaluation of the endometrium. Women at low risk of endometrial disease can be evaluated by ultrasonography on a less urgent basis. There is currently an increased interest for shifting a substantial number of hospital services to primary care providers. This may result in an increase of tests such as ultrasonography and endometrial biopsy being performed by general practitioners. This will lead to a large proportion of women with postmenopausal bleeding, mainly women at low risk of malignancy as identified by the predictive models, being investigated in primary care and thus avoiding referral to hospital services. Alternatively, if the DEFAB model is used, women at high risk of disease should be evaluated by direct visualisation of the endometrium and biopsy is dedicated setting that includes facilities such as outpatient hysteroscopy.

In Tables 3 and 4 we present the performance characteristics of the two predictive models at different cut-off values. Clinically it is important that the predictive model does not miss any patients with the disease. In order to achieve this the test should have high sensitivity and low false negative rate. However, as the sensitivity increases there is a decrease in the specificity of the test. There is no generally acceptable sensitivity and specificity value for the tests currently in use to evaluate women with postmenopausal vaginal bleeding. Even in cases where office-based endometrial biopsy is performed research has shown a 0.9% probability of endometrial disease [26]. It is therefore at the discretion of individual clinicians to decide which cut-off value to use for the predictive models depending on their practice, resources available and the prevalence of endometrial cancer in their local population. In order to determine if the predictive models can function in different groups of patients from where it was developed, there is need for external validation. We are currently completing the process of validating these two predictive models in a different setting of postmenopausal women using a different endometrial thickness cut-off value.

Several studies have proposed scoring systems for prediction of endometrial cancer [27,28]. The number of patients included in most of the studies was relatively small and the risk factors incorporated in each model varied. However, similarly to our studies the multivariate analyses from studies that developed scoring systems have incorporated increasing age, obesity and diabetes as significant risk factors in their predictive models.

One of the limitations of this paper is that the predictive models were developed based on the current clinical practice. Women found to have endometrial thickness measuring less than 5 mm on ultrasonography did not have tissue biopsy in order to exclude endometrial cancer. Consequently, the risk of false-negative cases is likely that have been underestimated. To estimate the false-negative rate, we searched our database to find out if any cases of women diagnosed with endometrial cancer were previously investigated for postmenopausal vaginal bleeding. We found that there were only three cases of patients with endometrial thickness measuring less than 5 mm on ultrasonography that subsequently were found to have malignancy. This gives a false-negative rate of 0.0015 (3/1893 cases). It is therefore unlikely that this would have any significant impact on the development of both predictive models.

In conclusion, in this manuscript, we have compared two clinical prediction models for endometrial cancer namely DEFAB and DFAB [12]. Of significant interest was to demonstrate whether these two prediction models can be used in place of the other depending on the available information on the well known predictors of endometrial cancer in particular, whether a clinician has an accurate measure of endometrial thickness or not. We have shown that for the proposed cut-off points for the DEFAB and DFAB tool, there was no evidence of a difference in discriminatory ability with respect to sensitivity, specificity, roc area, PPV and NPV. Further, we have excellent Kappa statistic indicating very good agreement. We have also verified the variables used in the predictive models namely diabetes, endometrial thickness, frequency of bleeding, age and BMI in classifying an individual being diagnosed with cancer or not through the logistic discriminant analysis approach. We recommend that these two predictive models can be used interchangeably.

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None required.

Conflict of interest

The authors have no conflict of interest to declare.

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To whom it may concern,

I confirm that Dr Nikolaos Burbos had the following contributions to the above manuscript:

1. The medical statistician, Dr P. Musonda, conceived the idea for the study.
2. Dr Burbos designed and database for the data collection and collected more than 75% of the data included in the study. He also had an oversight of the data collected by his colleagues.
3. A medical statistician carried out the analysis of the data. Dr Burbos arranged study group meetings to review the results, interpret and present the clinical significance of the findings.
4. Dr Burbos wrote the clinical aspect of the manuscript, incorporated changes from his co-authors and submitted the manuscript for publication. He responded to the comments made by the reviewers and submitted the revised version of the manuscript. Dr Musonda wrote the section about the statistical analysis on this manuscript.

Yours sincerely,

Joaquin J. Nieto

Chapter 5. Outcome of investigations for postmenopausal vaginal bleeding in women under the age of 50 years.

5.1 Introduction

In this chapter, I discuss the incidence of endometrial cancer diagnosis in young postmenopausal women. Data on the risk of endometrial cancer in young postmenopausal women are limited. The manuscript presented provides an estimation of the risk of endometrial cancer in a cohort of young women with postmenopausal vaginal bleeding (PMB).

5.2 Literature review

A large number of endometrial cancers are diagnosed in younger women. Felix et al performed a retrospective review of the data of 1752 patients diagnosed with endometrial cancer over a 12-year period and found that 18.3% of patients with type 1 and 8.5% of patients with type 2 tumours, were premenopausal [10]. In another study, Lee et al found that 2076 (4%) of a total of 51471 cases of endometrial cancer, were diagnosed in patients aged 16 to 40 years [170]. Soliman et al found that 12% of patients treated for endometrial cancer at a single institution were younger than 50 years [171].

Studies vary in the age selection criteria for defining 'young women'. Several authors used the threshold of 45 years [172-175], while other studies included only patients younger than 40 years that were diagnosed with endometrial cancer [40, 170, 176-178]. Other authors used an age threshold of younger than 50 years to define their cohort, as this criterion is used for hereditary cancer syndromes [171, 179]. The majority of patients diagnosed with endometrial cancer at younger age are obese and nulliparous [171, 175, 180, 181]. Other risk factors, such as a family history of endometrial cancer, age at first birth and use of hormonal preparations, have also been studied [179].

The risk of endometrial cancer in young women presenting with PMB has not been assessed adequately. The majority of the studies evaluating the risk of endometrial cancer in younger women included premenopausal patients only [171, 179, 182, 183]. Often the menopausal status of the patients is not reported [175, 181, 184], or when this information is provided, details on the clinical presentation of the patients were missing [180, 185, 186]. Further, studies evaluating the outcomes of investigations for patients with PMB, often excluded from the analysis women aged younger than 50 years [112, 113].

Only limited data exist on the risk of endometrial cancer in younger women with PMB. The authors of a prospective study evaluating 457 women with PMB, found no cases of endometrial cancer among 35 patients under the age of 50 years [84]. In another report, Evans-Metcalf et al reviewed the data on 40 patients, aged younger than 45 years, diagnosed with endometrial cancer [173]. The authors reported that one of the patients diagnosed with endometrial cancer presented with PMB. Thomas et al found that 25 of 138 patients diagnosed with endometrial cancer under the age of 45 years, were postmenopausal [180]. It is unclear, however, if any of these patients experienced PMB prior to the diagnosis. There were no data on the presenting symptoms of patients diagnosed with endometrial cancer on a previously published report of the same study [185]. In yet another study, Tran et al found 1 postmenopausal patient among 41 women diagnosed with endometrial cancer, aged younger than 45 years [186]. However, details on the clinical symptoms of patients diagnosed with endometrial cancer were not presented in the manuscript. The retrospective nature of the studies mentioned above is likely to account for the inconsistency and lack of data on this topic.

5.3 What does this study add?

This is the first study addressing the risk of endometrial cancer in young women presenting with PMB. This study suggests the risk of endometrial cancer in this group of patients is

significantly lower than in older postmenopausal women. The results of this study can be used to tailor the advice and investigation pathways for young women presenting with PMB.

5.4 What went well?

I conceived the idea for this study, wrote the manuscript, incorporated comments made by my co-authors and submitted the manuscript. I replied to comments made by the reviewers and submitted the revised version of the manuscript.

Prior to starting this research project, I prepared and submitted an ethics application to a national research committee and attended the interview process. This experience helped me to better understand the various ethical considerations in scientific research and the processes involved.

5.5 What could have been done differently?

In this study, we were unable to explain the reason for the low incidence of endometrial cancer in the younger group of women with PMB. It is likely that the age of the patient plays an important role in the risk of developing endometrial cancer after the menopause.

We also have not presented data on the long-term outcomes, including recurrence of vaginal bleeding and risk of endometrial cancer diagnosis, in this cohort of younger postmenopausal patients. This work is currently in progress and I aim to present the results in a new manuscript.

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Outcome of investigations for postmenopausal vaginal bleeding in women under the age of 50 years

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ABSTRACT

Objective. The objective of this study is to determine the incidence of endometrial cancer in young postmenopausal women presenting with vaginal bleeding.

Methods. Cross-sectional study of postmenopausal women presenting with vaginal bleeding in a gynaecological oncology centre in the United Kingdom. All women underwent transvaginal ultrasound scanning (TVS) as the initial investigation tool to evaluate the endometrium. Endometrial biopsy was performed only in cases where endometrial thickness measured equal to or greater than 5 mm. The patients were divided into two groups based on their age: less than 50 years (Group A) and 50 years or older (Group B).

Results. Over a 57-month period, 4454 women were investigated for postmenopausal vaginal bleeding. Of these, 259 (5.8%) women were diagnosed with endometrial carcinoma. 260 (5.8%) women were younger than 50 years. Endometrial biopsy was not performed in 130 women in Group A that had an endometrial thickness measurement of less than 5 mm on ultrasonography. With a median follow-up period of 3 (1–5) years, we found no cases of endometrial cancer in women under the age of 50 that did not undergo endometrial biopsy at the time of initial evaluation. Overall, no cases of endometrial cancer were diagnosed in postmenopausal women under the age of 50 years.

Conclusions. We found no cases of endometrial cancer amongst 260 women presenting with postmenopausal vaginal bleeding under the age of 50 years. These women could be investigated on a less urgent basis depending on the available resources.

Introduction

The average age of the menopause in the United Kingdom is 50 years and 9 months [1]. Early menopause (40–45 years) affects approximately 5% of women and premature ovarian failure (below the age of 40) is reported in 1% of women [2]. Postmenopausal vaginal bleeding is the main symptom of endometrial cancer, with 1–24% of women presenting with postmenopausal bleeding being diagnosed with endometrial malignancy [3–6].

The majority of cases of endometrial cancer are diagnosed in postmenopausal women [7]. However, up to 30% of cases are diagnosed in women younger than 50 years [7–10]. Several studies have investigated the clinical and pathologic characteristics in young women diagnosed with endometrial cancer. They are generally more obese and more likely to have a history of irregular menstrual cycles when compared to older women [7,11]. The definition "young women" varies between different studies: Gallup et al. reported data on women less than 40 years of age at the time of diagnosis [12]; other studies used cohorts of women younger than 45 years [8,13,14], and others used a cut-off of 50 years of age for their sample population [15,16]. The above studies focused mainly on premenopausal women and did not report the incidence of endometrial carcinoma in young postmenopausal women.

The aim of this study was to estimate the incidence of endometrial cancer in younger (less than 50 years) postmenopausal women presenting with vaginal bleeding and to determine the risk factors for this subgroup of women.

Methods

Between February 2006 and December 2010, 4454 postmenopausal women were referred for investigation of vaginal bleeding to a gynaecological cancer centre in the United Kingdom. All women underwent transvaginal ultrasound scanning as the initial investigation tool to evaluate the endometrium. The double wall endometrial

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thickness was measured in an anteroposterior dimension from one basalis layer to the other at its thickest part. In keeping with departmental guidelines, when the endometrial thickness measured less than 5 mm no further investigations were performed as evidence suggests a low probability of cancer below this threshold.

In this study we defined menopause clinically after at least 12 months of amenorrhoea. We appreciate the limitations with this approach, however biochemical confirmation (measurement of FSH, inhibin and/or estradiol levels) is also unreliable. We excluded from the study all premenopausal women, asymptomatic women with an incidental finding of increased endometrial thickness on radiological imaging, asymptomatic women with abnormal endometrial cytology found on cervical smear and women with a history of hysterectomy.

Women found to have an endometrial thickness equal to or greater than 5 mm had endometrial sampling performed using an endometrial Pipelle® device (Pipelle de Cornier; Laboratoire CCD, Paris, France). Endometrial biopsy was also performed in cases where the endometrial thickness was not clearly visualised on transvaginal ultrasonography. Hysteroscopic evaluation of the endometrium and biopsies were performed if a Pipelle® biopsy was not possible or did not yield sufficient tissue for histological diagnosis. Hysteroscopy was also performed in cases where endometrial thickness measurement by ultrasound was greater than 10 mm, in spite of benign histology on Pipelle® biopsy, when an endometrial polyp was suspected. We did not perform baseline endometrial biopsy in women with a history of breast cancer prior to commencing tamoxifen, as the evidence on this issue is conflicting. However, we investigate urgently women using tamoxifen if vaginal bleeding occurs.

We collected data regarding the presence of risk factors for endometrial cancer and the outcome of the investigations performed in a postmenopausal clinic database and analysed the data retrospectively. Recurrent episodes were defined as any episodes of bleeding lasting for 7 or more days or two or more separate bleeding events within the last 12 months prior to presentation to the general practitioner. Bleeding events that were previously investigated were not taken into account when classifying the episodes as single or recurrent. The patients were divided into two groups based on their age: less than 50 years (Group A) and 50 years or older (Group B).

The distributions of continuous variables were not symmetric. To test for normality, the Shapiro–Wilk W test was used, as was the q–q plot to investigate normality graphically (results not shown). There was no evidence to suggest that data was normally distributed, hence in the descriptive statistics for continuous variables, we report median and inter quartile range. To test any differences we used a non-parametric Wilcoxon rank sum (Mann–Whitney) test. Chi-squared test was used after checking the expected assumptions. All analyses were done using STATA software, version 10.1 SE (stata Corporation, Texas, USA).

Ethical approval for the use of the postmenopausal clinic database was granted by the National Research Ethics Service Committee South Central—Oxford C on the 29th of July 2011 (reference number: 11/SC/0285). The local Research and Development study number is 2011O&G06L (120-08-11).

Results

Over a 57-month period, 4454 women were investigated for postmenopausal vaginal bleeding. Of these, 259 (5.8%) women were diagnosed with endometrial carcinoma.

260 (5.8%) women were younger than 50 years; the mean age in this group was 47 years (group A). The remaining 4194 (94.2%) women were 50 years or older at the time of referral, with a mean age of 60 years in this group (group B).

The clinical characteristics of women in both groups are reported in Table 1. Significantly more women in group A were using HRT compared to group B (20.4% versus 15.3% respectively, $P<0.0001$). However the duration of HRT use was significantly longer in women in group B ($P<0.0001$). Similarly, more women in group A were using tamoxifen compared to group B (6.9% versus 3.3% respectively, $P<0.0001$). The mean duration of tamoxifen use was not different between the two groups. As expected, a significantly higher proportion of women that were 50 years or older were diagnosed with diabetes and hypertension when compared to younger women. In contrast, the proportion of women diagnosed with breast cancer was significantly larger in group A ($P<0.0001$).

Table 1
Clinical characteristics of women presenting with postmenopausal vaginal bleeding. §Two-sample Wilcoxon rank sum test (Mann–Whitney test). ‡Chi-squared test.

Clinical characteristics	Age groups		p-value
	<50 years (Group A)	≥50 years(Group B)	
Age, in years (IQR)	47 (46, 49)	60 (55, 68)	<0.0001§
BMI, median (IQR)	28 (24, 32)	28 (25, 33)	0.157§
Duration of HRT, in years	2 (1, 4)	5 (2, 10)	0.0001§
Tamoxifen use, in years	3 (2, 3)	3 (2, 5)	0.085
Amount of bleeding			
Spotting	27 (11%)	771 (19%)	<0.0001‡
Light	123 (48%)	2550 (62%)	
Heavy	107 (42%)	785 (19%)	
Frequency of bleeding			
Single Episode	144 (56%)	2060 (49%)	0.042‡
Recurrent	114 (44%)	2119 (51%)	
Diabetes			
No	253 (97%)	3934 (94%)	0.021‡
Yes	7 (3%)	260 (6%)	
Hypertension			
No	245 (94%)	3045 (73%)	<0.0001‡
Yes	15 (6%)	1149 (27%)	
Breast cancer			
No	228 (88%)	3922 (94%)	<0.0001‡
Yes	32 (12%)	272 (6%)	
Number of patients (%)	260 (5.8)	4194 (94.2)	

The results of investigations for both groups of postmenopausal women are shown in Table 2. No cases of endometrial cancer were diagnosed in postmenopausal women under the age of 50 years. There was no significant difference in the proportion of women with endometrial thickness measurement of equal to or greater than 5 mm between the two groups. The majority of women in group B had an endometrial thickness measurement less than 5 mm on ultrasound and hence no further investigation was required. Significantly more women in group A were found to have normal endometrium when biopsy was performed and significantly more women in group B were found to have endometrial polyps.

Only 3 cases of endometrial hyperplasia were diagnosed in women under the age of 50 years. The histology showed simple hyperplasia without atypia in all of the cases. In the group of women aged 50 years or older there were 74 cases of endometrial hyperplasia. In this group histology showed 49 (66.2%) cases of simple hyperplasia without atypia, 6 (8.1%) cases of complex hyperplasia, 2 (2.7%)

Table 2
Outcome of investigations in women presenting with postmenopausal vaginal bleeding. ‡Chi-squared test.

Outcome of investigations	Age groups		*P* value
	<50 years (Group A) n (%)	≥50 years(Group B) n (%)	
ET<5 mm	130 (50%)	1933 (46.1%)	0.220
Normal histology	116 (44.6%)	1411 (33.6%)	<0.0001‡
Endometrial polyps	9 (3.5%)	461 (11.0%)	<0.0001‡
Endometrial hyperplasia	3 (1.2%)	74 (1.8%)	0.464
Endometrial cancer	0 (0%)	259 (6.2%)	<0.0001‡
Other	2 (0.7%)	56 (1.3%)	0.435

cases of simple hyperplasia with atypia and 17 (22.9%) cases of complex atypical hyperplasia. Amongst women with the diagnosis of complex atypical hyperplasia, 7 underwent hysterectomy and malignancy was found in only one case. One patient diagnosed initially with complex atypical hyperplasia presented 3 years later with postmenopausal bleeding and investigation showed grade 1 endometrial adenocarcinoma. 9 women with complex atypical hyperplasia were managed medically using progestogens and no cases of cancer were diagnosed with average follow-up of 1.6 years.

Conclusions

Increasing age is a risk factor for development of endometrial cancer. The median age at diagnosis is 63 years [17]. Although a significant proportion of uterine cancer cases are diagnosed in women under the age of 50 years [7,10], the incidence of endometrial carcinoma in young postmenopausal women has not been previously reported. Evans-Metcalf et al. reported data showing that 40 (13.8%) of the patients treated with endometrial cancer at their institute were younger than 45 years [8]. Only one patient in the group of women under the age of 45 years was postmenopausal. Schmeler et al. found that 188 (12%) of cases of endometrial cancer in their study occurred in premenopausal women and under the age of 50 years at the time of diagnosis [7]. No cases of endometrial cancer were reported in young postmenopausal women in their study. Parslov et al. reported data on a large cohort of women under the age of 50 years diagnosed with endometrial cancer but did not include postmenopausal women in their study [15]. However, Thomas et al. reported in their cohort of women with endometrial cancer 25 cases of malignancy in postmenopausal patients under the age of 45 years [18]. The authors of the above study acknowledge that the data about menstrual status were collected in the early 1980s and the measures about menstrual history were self-reported, and may therefore be unreliable [18]. Similarly in our study, we did not include the time since menopause as a variable as we found that women's ability to recall the time of last menstrual period was not reliable or accurate. Some authors suggest the time since menopause is an important factor in diagnosing endometrial cancer in women with postmenopausal vaginal bleeding [19]. However, it is likely that the increasing time since menopause may simply be a confounding variable for age.

In our study, we found no cases of endometrial cancer in a cohort of 260 women presenting with postmenopausal vaginal bleeding under the age of 50 years. The greatest strengths of this study are the sample size and large number of women with postmenopausal vaginal bleeding under the age of 50 years. Reporting data from such a large cohort of young postmenopausal women is partly facilitated by the national recommendations in the United Kingdom according to which, all postmenopausal women presenting with vaginal bleeding should be referred to secondary care for further investigation regardless of their age in order to exclude malignancy [20]. In our study, older women were more likely to have the endometrial cancer risk factors of diabetes and/or hypertension compared to women under the age of 50 years. We found no significant difference in the body mass index between the two groups of women studied. However, despite the higher incidence of hypertension, diabetes and recurrent episodes of vaginal bleeding in women aged 50 years or older, one would expect that cases of malignancy would be present in younger postmenopausal women in such a large cohort.

One of the limitations of this study is that we used transvaginal ultrasonography for the initial assessment of women presenting with postmenopausal vaginal bleeding. For women with endometrial thickness measuring less than 5 mm, endometrial biopsy was not performed. This practice was based on published guidelines and evidence suggesting low probability of endometrial cancer in this group of women [21,22]. However, more recent evidence suggests that a lower threshold for endometrial thickness measurement of 3 mm should be used for exclusion of endometrial cancer in women presenting with postmenopausal vaginal bleeding [23]. We interrogated our postmenopausal bleeding clinic database and pathology database and with a median follow up of 3 (1–5) years, found no cases of endometrial cancer in women under the age of 50 years and an endometrial thickness measurement of less than 5 mm at the time of initial evaluation. In addition, the geographic area that our cancer centre covers is characterised by particularly stable population, hence it is unlikely that cases of malignancy in this group were diagnosed elsewhere.

The main objective of this study was to evaluate the risk of endometrial cancer in young, symptomatic postmenopausal women. Although less frequently, endometrial cancer is also diagnosed in premenopausal women. However, in this manuscript we did not report data about the incidence of endometrial cancer in premenopausal women. The criteria for investigation of premenopausal women presenting with irregular vaginal bleeding to exclude endometrial cancer vary widely between different institutes and different clinicians and this does not allow for an accurate estimation of incidence of endometrial cancer in this group of women.

In summary, our study reports the outcome of investigation of a large cohort of symptomatic postmenopausal women aged younger than 50 years that were investigated at a single institution. We found no cases of endometrial cancer amongst 260 women investigated for postmenopausal vaginal bleeding. Based on these findings we would recommend that women presenting with postmenopausal vaginal bleeding under the age of 50 years, could be investigated on a less urgent basis depending on the available resources. This may include measurement of endometrial thickness using TVS in the primary care setting and referral to hospital clinics only for women with endometrial thickness measurement of equal to or greater than 4 or 5 mm. Alternatively, implementation of a risk stratification system using clinical prediction models that incorporate patient's age as a variable, may improve diagnostic pathways for women with postmenopausal vaginal bleeding [24,25].

Conflict of interest statement

The authors have no conflict of interest to declare.

Disclosure

The data presented in this manuscript are part of an ongoing project for evaluation and improvement of diagnostic pathways for women with postmenopausal vaginal bleeding.

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Burbos N, Musonda P, Crocker SG, Morris EP, Duncan TJ, Nieto JJ (2012) **Outcome of investigations for postmenopausal vaginal bleeding in women under the age of 50 years.** Gynecol Oncol 125 (1):120-123. doi:10.1016/j.ygyno.2011.12.453

To whom it may concern,

I confirm that Dr Nikolaos Burbos had the following contributions to the above manuscript:

1. He conceived the idea for the study.
2. He designed and database for the data collection and collected more than 75% of the data included in the study. He also had an oversight of the data collected by his colleagues.
3. A medical statistician carried out the analysis of the data. Dr Burbos arranged study group meetings to plan the analysis, review the results, interpret and present the clinical significance of the findings.
4. He wrote the manuscript, incorporated changes from his co-authors and submitted the manuscript for publication. He responded to the comments made by the reviewers and submitted the revised version of the manuscript.

Yours sincerely,

Joaquin J. Nieto

Chapter 6. Age-related differential diagnosis of vaginal bleeding in postmenopausal women: a series of 3047 symptomatic postmenopausal women.

6.1 Introduction

The differential diagnosis of postmenopausal vaginal bleeding (PMB) varies depending on the patient's age. Evaluation of patients with PMB requires a good understanding of the various causes of the symptom. Most of the data on the outcomes of investigations for women with PMB are based on older reports that do not reflect changing population demographics. In this chapter, I present a study conducted to determine the age-related differential diagnosis of PMB in a geographical region in the United Kingdom.

6.2 Literature review

The incidence of postmenopausal vaginal bleeding (PMB) is inversely related to the time elapsed since menopause [81]. Parker et al analysed the data of 10122 patients with PMB, included in the primary care research database in the United Kingdom [85]. The authors found that the rate of consultations for PMB in primary care was higher in women aged 55-59 years and lowest in the group of patients older than 85 years (7.4/1000 per year versus 1.5/1000 per year, respectively), while the risk of endometrial cancer was approximately 13 times lower in the age group from 55-59 years compared to patients aged 75 years or older [85]. Gredmark et al found the peak incidence of endometrial cancer (87/100000 women years) among patients investigated for PMB is observed in the age group from 65 – 69 years [84].

Lower genital tract and endometrial atrophy is the most common finding in women investigated for PMB [100, 111, 187-190]. The reported rate of genital tract atrophy among women with PMB varies between 40% to 83% [84, 108, 111, 112, 187-189]. The observed variation in the incidence of genital tract atrophy among women with PMB reflects the biases

in patient selection in different studies and the heterogenous ultrasonographic, histological or clinical criteria used to define endometrial atrophy.

Endometrial polyps and fibroids (or leiomyomas) are frequently reported findings in women investigated for PMB. The majority of endometrial polyps are benign [191, 192]. The incidence of endometrial polyps among women with PMB varies between 2% to 42% [190, 193-196]. The rate of endometrial polyps was higher in studies where hysteroscopy was used as the reference test [194, 195] and lower in studies where curettage alone was used to obtain a histological diagnosis [111, 187, 193]. There is also evidence that the use of hysterosonography improves the detection of endometrial polyps in women with PMB [136]. The incidence of uterine fibroids in women with PMB is mainly reported in studies where hysteroscopy was performed as part of the investigations. The percentage of patients that are found to have fibroids during investigations for PMB varies between 8% to 16% [190, 194, 196, 197].

Other malignancies such as ovarian, cervical or vulval carcinomas are less frequently diagnosed in women presenting with PMB. In a case-control study, the positive predictive value of PMB for ovarian cancer was estimated to be 0.5% (95% CI 0.2 - 0.9) [198]. The incidence of cervical cancer in patients with PMB varies widely in the literature. In a study of 1019 women investigated for PMB between the years of 1969 and 1972, the authors found 22.4% of the patients had cervical cancer [199]. Among patients diagnosed with cervical cancer in this study, 31% were found to have advanced disease. However, more recent studies report a lower rate of cervical cancer in women investigated for PMB varying between 1.5% to 5% [114, 200].

Changing trends have been observed in the prevalence of risk factors for endometrial cancer in the population over time. For example, the average age of the population in the UK is increasing [201]. As a consequence, a greater proportion of the population is affected by

multimorbidity, including diabetes [202]. In addition, the rates of obesity in women have increased and the rise is expected to continue [203]. These changes are likely to affect the background prevalence of endometrial cancer among women with PMB. Hence, up to date studies are required to evaluate the outcomes of investigations for women with PMB.

6.3 What does this study add?

This study investigated and presents the causes of PMB by age group. The results of the study can be useful during clinical consultations with women with PMB, to discuss the differential diagnosis.

6.4 What went well?

This is one of the first studies that I published using data collected in a database I personally developed. After some time, I realised that the database had several drawbacks. The main issues were related to the ease of data entry and problems with extraction of datasets for analysis. To overcome these problems, I read books and watched videos on data preparation and coding. Collaboration with the statistician allowed me to simplify the recording of the data and to make the process of data extraction more time-efficient. I also had regular meetings with my colleagues working on this project to review the quality and consistency of data collection. These discussions helped to improve the design of the database further. I also developed a better understanding of options on data storage, encryption and anonymisation.

6.5 What could have been done differently?

The denominator for calculating the incidence of PMB and endometrial cancer in the population was the number of women living in Norfolk County at the time of the study. We were unable to identify and exclude from the data patients that may have previously undergone hysterectomy for any gynaecological indications, including cases of previously treated endometrial cancer. Excluding these patients from the calculations would have provided more precise estimation of the relevant incidence risk.

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Original article

Age-related differential diagnosis of vaginal bleeding in postmenopausal women: a series of 3047 symptomatic postmenopausal women

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Abstract

Objective. The aim of this study is to identify the causes of vaginal bleeding in different age groups of postmenopausal women. Also, we attempt to estimate the incidence of postmenopausal vaginal bleeding and endometrial cancer in a defined geographical area.

Study design. The study was conducted at a gynaecological oncology centre in the United Kingdom, between February 2006 and May 2009. Patients were investigated according to established evidence-based departmental guidelines.

Results. During the study period 3047 women were referred with postmenopausal vaginal bleeding. In 1356 women (44.5%) the endometrial thickness measured less than 5 mm on transvaginal ultrasound scan. Benign histology was found in 1144 women (37.5%). Benign endometrial polyps were the cause of bleeding in 10.1% of the cases. The incidence of endometrial cancer in our study population was 5%. The rate of postmenopausal vaginal bleeding during the study period peaks at the age of 55–59 years (25.9/1000 postmenopausal women/year) and declines thereafter. The peak incidence of endometrial cancer during the study period (12.6/10,000 postmenopausal women/year) was seen between the ages of 60 and 64 years and similarly declines with increasing age.

Conclusion. To our knowledge, this is the first population-based estimation of the incidence of genital tract bleeding and endometrial cancer among postmenopausal women in the United Kingdom. The results of this study showing the age-related differential diagnosis can be used to inform clinical practice when counselling postmenopausal women with vaginal bleeding.

Keywords: Incidence, postmenopausal bleeding, endometrial cancer

Introduction

Postmenopausal vaginal bleeding is one of the most common indications for presentation to a gynaecological clinic. The estimated incidence of bleeding immediately after the first 12 months of amenorrhea following the menopause is 409/1000 person-years, falling to 42/1000 person-years more than three years after menopause.[1] The differential diagnosis of bleeding in postmenopausal women is narrower than that of abnormal bleeding in premenopausal women due to the lack of the variable influence of ovarian hormones. Abnormal vaginal bleeding in the postmenopausal years is usually attributed to an intrauterine source, but may also arise from the cervix, vagina, vulva, fallopian tubes or be related to ovarian pathology. The origin of bleeding can also involve non-gynaecological sites, such as the urethra, bladder and lower gastrointestinal tract. The primary aim when investigating women with postmenopausal bleeding is to exclude endometrial malignancy and any significant additional abnormalities.

The reported incidence of endometrial carcinoma in women presenting with postmenopausal vaginal bleeding varies widely between different studies, from 1% to

24%.[2–9] It is acceptable for the incidence of cancer to vary between different populations, depending on the presence of risk factors for endometrial malignancy. However, of the studies conducted so far, few were population based and selection bias may be responsible for the wide variation in the incidence of cancer. This degree of selection bias may be less marked in studies conducted in the United Kingdom, where robust guidelines govern the referral of women with postmenopausal vaginal bleeding to secondary care in order to exclude endometrial cancer.[10]

The incidence of the risk factors for endometrial carcinoma is changing as the overall population is becoming older with conditions such as diabetes, hypertension and obesity becoming more prevalent. This behoves a need for studies to reflect the changes in patients' characteristics and structure of the population.

The aim of this study is to identify the causes of vaginal bleeding in different age groups of postmenopausal women. Also, we attempt to estimate the incidence of postmenopausal vaginal bleeding and endometrial cancer in a defined geographical area.

Materials and methods

The study was a prospective cohort study, conducted at a gynaecological oncology centre in the United Kingdom, between February 2006 and May 2009. All postmenopausal women presenting with vaginal bleeding were included. All the data analysed were collected from departmental proformas kept as part of routine investigations and treatment. Patients were investigated according to established evidence-based departmental guidelines.

Menopause was defined as at least 12 months of spontaneous amenorrhoea. Premenopausal women were not included in the study as there is no standard threshold for endometrial thickness in this group that is considered abnormal. Other groups of women seen at the clinic that were excluded from the study included asymptomatic women with an incidental finding of increased endometrial thickness on imaging and asymptomatic women with abnormal endometrial cytology found on cervical smear.

All women presenting with vaginal bleeding underwent transvaginal ultrasound scanning to evaluate the endometrium as part of their routine assessment. The double wall endometrial thickness was measured in an anteroposterior dimension from one basalis layer to the other. When endometrial thickness measured less than 5 mm, according to the departmental guidelines, no further investigations were performed as evidence suggests a low probability of cancer below this threshold.[11,12]

Women found to have endometrial thickness equal to or greater than 5 mm had endometrial sampling performed using an endometrial Pipelle® device. Hysteroscopic evaluation of the endometrium with biopsy under a general anaesthetic was performed if Pipelle® biopsy was not possible or did not yield sufficient tissue for histological diagnosis. A general physical and gynaecological examination was performed in order to exclude other causes of vaginal bleeding.

The results recorded were divided into the following groups: atrophy (including women with endometrial thickness measurement of less than 5 mm), benign histology, benign endometrial polyps, endometrial hyperplasia, endometritis, endometrial carcinoma and other conditions (including cervical cancer or bladder carcinoma).

The incidence of postmenopausal vaginal bleeding and endometrial cancer in our population was calculated using local population statistics for the year 2007;[13] the denominator for the calculations was the female population living in Norfolk county, by five-year age cohorts.

We used Stata statistical software version 10.1 SE (Stata Corporation, College Station, TX, USA). We set statistical significance at two-sided $P < 0.05$. Descriptive statistics were calculated as simple tabulations of frequencies and percentages. Binomial exact test was used to calculate the 95% confidence intervals of the percentages. To test any associations between age categories and endometrial cancer diagnosis, Fisher's exact test was used. A test of linear trend developed by Cuzick[14] was used to test whether increasing age was associated with endometrial cancer or other known risk factors. Kruskal–Wallis test was used to test any differences in the duration of hormone replacement therapy (HRT) in each age group.

Results

Table 1 shows the frequency of the different causes of vaginal bleeding in our study population. For women with endometrial thickness measurement of less than 5 mm on transvaginal ultrasound scan, the symptoms were attributed to genital tract atrophy following a negative clinical examination. Table 2 shows the underlying cause of bleeding in different age groups of women. Using Fisher's exact test, there was strong evidence ($P < 0.0001$) of an association between endometrial cancer diagnosis and age. The test for linear trend was statistically significant ($P = 0.034$) (see Figure 1). In the 11 women who comprise the 'other conditions' group, the histological diagnoses were as follows: metaplasia (four cases), bladder carcinoma (three cases), cervical carcinoma (two cases) and ovarian carcinoma (two cases).

There were 488 (16%) women taking HRT. We included this group of women in our analysis in order to avoid selection bias. All women were taking combined regimens of HRT. Our analysis showed that duration of HRT use did not affect the risk of developing endometrial carcinoma. Only nine of 488 (1.8%) women using HRT developed endometrial carcinoma.

Using local population statistics for Norfolk for the year 2007, we estimated the incidence of vaginal bleeding and endometrial cancer in postmenopausal women by five-year age cohorts. The incidence of postmenopausal vaginal bleeding in our population peaks at the age of 55–59 years and declines thereafter. The peak incidence of endometrial cancer is seen in the age group 60–64 years (Table 3). Type I endometrial cancer (endometrioid histology) comprises 86% of the cases, while type II cancers (non-endometrioid histology) account for 14% of the endometrial tumours diagnosed during the study period.

Table 1 Frequency of the observations

Outcome	Frequency	Percentage	95% CI
Atrophy	1356	44.50	42.72–46.28
Benign histology	1144	37.55	35.82–39.29
Benign endometrial polyps	309	10.14	9.09–11.26
Endometrial hyperplasia	62	2.03	1.56–2.60
Endometritis	16	0.53	0.30–0.85
Type I endometrial carcinoma	128	4.20	35.16–49.74
Type II endometrial carcinoma	21	0.69	0.45–1.05
Other conditions	11	0.36	0.20–0.64
Total	3047	100.00	

CI, confidence interval

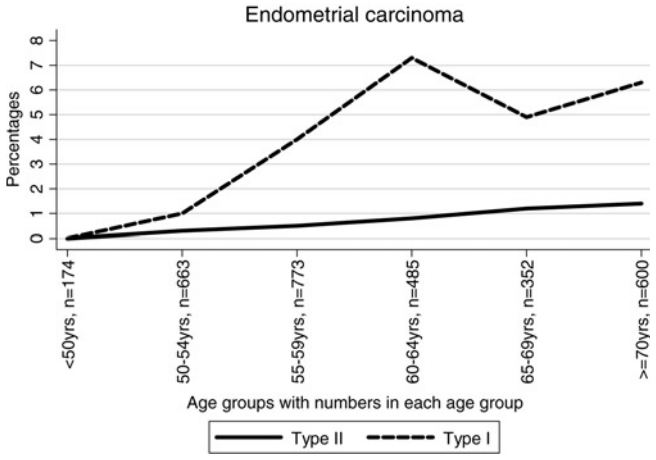

Figure 1 Percentage of endometrial cancer as a cause for abnormal genital tract bleeding in the different age groups of postmenopausal women

Discussion

Our study reports on the outcome of investigations on a large cohort of postmenopausal women presenting with vaginal bleeding. In the United Kingdom, postmenopausal women with vaginal bleeding are referred by the general practitioner to secondary care for further investigations in order to exclude malignancy, within two weeks of the initial presentation. We performed analysis of the routinely collected data for the referrals and their outcome over a 39-month period, which helps to minimize selection bias in the study sample.

The reported incidence of endometrial carcinoma varies between different studies. The incidence of endometrial carcinoma reported by Gambrell *et al.*[4] was 1.5%, while Alberico *et al.*[7] found the incidence of endometrial carcinoma and hyperplasia in their study to be 24.4%. Iatrakis *et al.*[9] reported an 11.1% incidence of cancer in their study; Choo *et al.*[5] and Lidor *et al.*[6] found a similar rate of carcinoma of 7%. In these studies there was variation in the selection criteria used and also the prevalence of risk factors for endometrial carcinoma. This may have contributed to the wide range in the observed incidence of endometrial carcinoma.

In our study we report a 5% incidence of endometrial cancer. This relatively low incidence may reflect the strict referral criteria for women with postmenopausal vaginal bleeding, which effectively lead to all women presenting to their general practitioner with this symptom being referred to secondary care. The incidence of endometrial cancer in our population peaks in the age group of 60–64 years. The peak incidence of cancer found by Gredmark *et al.*[8] in their study was in the age interval of 65–69 years. The population-based incidence of endometrial cancer in our study appears to be lower than in the study by Gredmark *et al.* for each age group after the age of 50 years.

By using population data as the denominator for the estimation of the incidence of bleeding and endometrial cancer, we made an assumption about the catchment area for our clinic. Norfolk is a well-defined geographical region with relatively minor changes of the population over time, but women are given the option of being seen in hospitals outside the region (under the 'Choose and Book' system). This may be balanced by similar referrals from outside the region that were seen in our clinic.

In our study, the group under the age of 50 years included women from the age of 35–49 years who were postmenopausal. However, we did not have a way of correctly identifying the total number of postmenopausal women under the age of 50 in the catchment area population. This has resulted in the use of an overestimated denominator and thus significant underestimation of the incidence of bleeding in this group of women. However, this does not affect the incidence of cancer in the population group as no cancers were found under the age of 50 years.

Many postmenopausal women who take HRT will have vaginal bleeding as result of their treatment. However, in order to avoid selection bias we included this subgroup in our analysis. The low rate of endometrial cancer in the HRT group can also contribute to the relatively low

Table 2 Frequency of outcomes in different age groups

Histology	Age group (years)					
	<50	50–54	55–59	60–64	65–69	≥70
Atrophy	77 (44.3%)	285 (43%)	336 (43.4%)	198 (40.8%)	174 (49.4%)	286 (47.7%)
Benign histology	89 (51.2%)	310 (46.7%)	312 (40.4%)	167 (34.5%)	96 (27.3%)	170 (28.3%)
Endometrial polyps	6 (3.4%)	51 (7.7%)	65 (8.4%)	67 (13.8%)	44 (12.5%)	76 (12.7%)
Endometrial hyperplasia	2 (1.1%)	7 (1.0%)	21 (2.7%)	12 (2.5%)	10 (2.8%)	10 (1.7%)
Endometritis	0	1 (0.2%)	3 (0.4%)	1 (0.2%)	4 (1.2%)	7 (1.1%)
Type I endometrial carcinoma	0	7 (1.0%)	30 (3.9%)	36 (7.4%)	16 (4.5%)	39 (6.5%)
Type II endometrial carcinoma	0	1 (0.2%)	3 (0.4%)	3 (0.6%)	5 (1.4%)	9 (1.5%)
Other conditions	0	1 (0.2%)	3 (0.4%)	1 (0.2%)	3 (0.9%)	3 (0.5%)
Total	174 (100%)	663 (100%)	773 (100%)	485 (100%)	352 (100%)	600 (100%)

Table 3 Rate of postmenopausal vaginal bleeding and endometrial cancer in a defined geographical area during the study period

Age groups (years)	Total number of cancers in 30-month study period	Women with PMB in each age group	Women in each age group in Norfolk	Rate of bleeding during the 39-month study period = 10.4 (10.4–10.78)	Rate of endometrial cancer during the 39-month study period = 5.1 (4.30–5.97)
<50	0	174	109,778	1.6 (1.4–1.8)	0 (0–3)
50–54	8	663	27,154	24.4 (22.6–26.3)	2.9 (1.3–5.8)
55–59	33	773	29,775	25.9 (24.2–27.8)	11.1 (7.6–15.6)
60–64	39	485	30,849	15.7 (14.3–17.1)	12.6 (9.2–17.3)
65–69	21	352	23,588	14.9 (13.4–16.6)	8.9 (5.8–13.6)
≥70	48	600	71,624	8.3 (7.7–9.0)	6.7 (4.9–8.9)
Total	149	3047	292,768	10.4	5.1

PMB, postmenopausal bleeding; CI, confidence interval
Incidence and 95% confidence intervals were calculated using Binomial exact methods

incidence of endometrial cancer in our study population compared with previous publications. Our results are in agreement with data from the Women's Health Initiative randomized trial that found no significant difference in the risk of endometrial cancer between women on combined HRT preparations and placebo.[15]

Similar to the study by Gredmark *et al.*, the incidence of cervical cancer was very low. Only two cases of cervical carcinoma were diagnosed in our study. As in the above study this is most likely a reflection of the effective national cervical screening programme.

One of the limitations of our study is the lack of histological confirmation for the exclusion of endometrial malignancy in the group of women where the endometrium measured less than 5 mm on ultrasound scan. This group of women comprised 44.5% of the cases in our study. The current practice in our unit not to perform endometrial biopsy is based on evidence suggesting low probability of endometrial cancer in this group,[12] and for the purposes of the study, if the clinical examination did not reveal any other pathology then the symptoms were attributed to genital tract atrophy. However, if we consider a 1% post-test probability of malignancy for women with endometrial thickness measurement of less than 5 mm,[12] then 13 additional cases of endometrial cancer should be included in the results. Thus, the incidence of cancer in our study would then change from 5% to 5.3%. Although this is small change in the overall incidence, it may have an effect in the peak incidence depending on the age group distribution of the additional cases of cancer.

To our knowledge, this is the first population-based estimation of the incidence of genital tract bleeding and endometrial cancer among postmenopausal women in the United Kingdom. The results of this study showing the age-related differential diagnosis can be used to inform clinical practice when counselling postmenopausal women with vaginal bleeding.

Peer review was directed independently of the authors by Dr Heather Currie, co-editor of *Menopause International*.

Competing interests: None declared.

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To whom it may concern,

I confirm that Dr Nikolaos Burbos had the following contributions to the above manuscript:

1. He conceived the idea for the study.
2. He designed and database for the data collection and collected more than 75% of the data included in the study. He also had an oversight of the data collected by his colleagues.
3. A medical statistician carried out the analysis of the data. Dr Burbos arranged study group meetings to plan the analysis, review the results, interpret and present the clinical significance of the findings.
4. He wrote the manuscript, incorporated changes from his co-authors and submitted the manuscript for publication. He responded to the comments made by the reviewers and submitted the revised version of the manuscript.

Yours sincerely,

Joaquin J. Nieto

Chapter 7. Postmenopausal vaginal bleeding in women using hormone replacement therapy.

7.1 Introduction

Postmenopausal women using hormone therapy that experience unscheduled vaginal bleeding, require investigations. The risk of endometrial cancer in this group of patients is not clearly evaluated, as they have often been excluded from relevant studies. In this chapter, I present a study conducted to investigate the causes of postmenopausal vaginal bleeding (PMB) in women using postmenopausal hormone therapy.

7.2 Literature review

Oestrogen is the most effective treatment for management of symptoms associated with menopause [204-206]. However, the use of oestrogens is associated with an increased risk of endometrial hyperplasia and cancer [207, 208]. Meuwissen et al reported that women develop endometrial hyperplasia after using unopposed oestrogens for an average duration of 186 days [6]. A meta-analysis of 29 observational studies reported that the risk of endometrial cancer is significantly higher in women using unopposed oestrogen therapy compared to never users (RR 2.3, 95% CI 2.1-2.5) [208]. The authors of this study found that the risk of endometrial cancer in women using oestrogen-only hormone therapy is increased with longer duration of use. Similar results were reported by more recent studies [207, 209]. Addition of progestins decreases the risk of endometrial hyperplasia or cancer associated with oestrogen use [80]. Progestins can be given continuously or in cyclical fashion for a certain duration each month, or 3-monthly. In a meta-analysis of 7 studies, the relative risk of endometrial cancer in women using combined oestrogen and progestin preparations compared to non-users, was 0.8 (95% CI 0.6-12 [208]. Similar results were reported by the authors of the Women's Health Initiative (WHI) randomised trial, that found the hazard ratio for endometrial cancer in women using combined hormone therapy preparation compared to

placebo, was 0.81 (95% CI, 0.48-1.36) [83]. Other reports suggest that a reduction in the risk of endometrial cancer is not observed in patients using cyclical progestins [210]. In a large cross sectional study, monthly or every 3-months addition of progestin, was found to increase the risk of endometrial cancer in patients receiving oestrogen preparations compared to the general population [211]. In another report, the risk of endometrial cancer was higher in patients using oestrogen therapy, when progestins were added for 10 or less days each cycle (odds ratio 2.9, 95% CI 1.8-4.6) [207].

The vaginal bleeding pattern in women using postmenopausal hormone therapy varies depending on the type of regimen used. Approximately 80% of women using cyclical postmenopausal hormone therapy will experience regular withdrawal bleeding, while the rate of amenorrhoea for women using continuous combined preparations is greater than 70% [82]. The likelihood of irregular vaginal bleeding is reduced as the interval from the time of menopause increases [82].

Currently there is no consensus on how to investigate women with PMB that are taking postmenopausal hormone therapy. TVUS is not considered adequately safe to monitor women receiving estrogen-only hormone therapy, to predict the risk of endometrial hyperplasia or cancer [6]. Among postmenopausal women that required a TVUS in the WHI study, there was no significant difference in the percentage of patients with a thin endometrial stripe (endometrial thickness ≤ 5mm) in the group receiving oestrogen and progestin compared to the group receiving placebo (70.8% versus 75% respectively; p =0.16) [83]. Omodei et al found that 57% of women that experienced PMB while on postmenopausal hormone therapy had an endometrial thickness measuring ≤4.5 mm on TVUS [212].

The most common histological findings in biopsies from women receiving cyclical hormone therapy show secretory and proliferative endometrium, while the use of continuous combined hormone preparations induces atrophic changes [213]. The rate of benign endometrial polyps

or fibroids is not significantly different between women taking cyclical regimens and those on continuous combined hormone preparations [190]. However, data on the differential diagnosis for women with PMB that are taking postmenopausal hormone therapy are lacking.

7.3 What does this study add?

The results of this study provide an understanding of the risk of endometrial cancer in a large cohort of women that use combined hormone therapy and present with PMB. The proportion of women using postmenopausal hormone therapy found to have a thin endometrium on TVUS is lower than previously reported.

7.4 What went well?

The lack of clear guidance on the investigation pathways for women with PMB using hormone therapy triggered my interest to start working on this manuscript. Following discussion with my co-authors and the statistician, we carefully planned the presentation of results relevant to clinical practice. I wrote and submitted the manuscript for publication.

Working on this project again emphasised the importance of teamwork in research. Complimentary knowledge from colleagues with significant previous research experience on the management of menopausal symptoms helped me to shape the direction of the manuscript. It also helped with the review of literature, interpretation of the results and to better understand the limitations of this work.

7.5 What could have been done differently?

The main limitation of this study is the lack of information on the type of hormone therapy preparation that the patients used. Although all the patients in the study used oestrogen plus progestin preparations, we do not have data to confirm if these were continuous or sequential regimens. Also, for practical reasons, in patients taking sequential hormone therapy, we were unable to schedule the TVUS assessment of the endometrium in the early cycle.

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Original article

Postmenopausal vaginal bleeding in women using hormone replacement therapy

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Abstract

Objective. To estimate the risk of endometrial cancer in postmenopausal women presenting with vaginal bleeding using estrogen–progestogen hormone replacement therapy (HRT) regimens and to assess if the duration of HRT use has an effect on the risk of diagnosing endometrial cancer.

Study design. Cross-sectional study of consecutive women presenting with postmenopausal vaginal bleeding at a gynaecological oncology centre in the UK.

Main outcome measures. Endometrial cancer diagnosis.

Results. Over a 62-month period, 4847 women were investigated for postmenopausal vaginal bleeding. The majority of women (4097, 84.5%) did not use any HRT preparation at the time of initial referral and 750 (15.5%) women were using combined HRT preparations. A total of 298 (6.1%) women were diagnosed with endometrial carcinoma. Women using HRT preparations were significantly less likely to be diagnosed with endometrial cancer compared with women not using HRT (adjusted odds ratio = 0.229, 95% CI 0.116–0.452; $P < 0.0001$). The longer duration of HRT use did increase the risk of diagnosing endometrial cancer in women presenting with postmenopausal vaginal bleeding, but this was not statistically significant.

Conclusions. Postmenopausal women presenting with vaginal bleeding and using combined HRT preparations have significantly lower risk of being diagnosed with endometrial cancer when compared with women not using HRT.

Keywords: Postmenopausal bleeding, hormone replacement therapy, endometrial cancer

Introduction

According to national recommendations, any episodes of vaginal bleeding in postmenopausal women other than the monthly withdrawal bleeding with cyclical combined hormone replacement therapy (HRT) should be investigated in order to exclude malignancy.[1] The most common type of malignancy in this group of women is endometrial cancer; the reported risk in literature varies from 1% to 24%.[2–5]

Type I endometrial carcinoma is estrogen-related and associated with the presence of risk factors such as obesity, diabetes, nulliparity and unopposed estrogen stimulation.[6] The risk of developing endometrial carcinoma in women using estrogen-only HRT has been shown to vary between 2% and 15%.[7,8] Available data suggest that the risk of endometrial cancer in postmenopausal women using HRT is largely reduced by addition of progestogens in either continuous or cyclical regimens.[9–12]

Up to 90% of women using cyclical combined HRT preparations will experience monthly withdrawal bleeding.[13] In contrast, continuous administration of progestogens will lead to endometrial atrophy and amenorrhoea.[14] However, this phase of amenorrhoea may be preceded by an initial period of irregular vaginal bleeding. This fact is often disregarded and women using continuous combined HRT preparations for less than six months are referred for investigation of postmenopausal vaginal bleeding.

Studies on assessing the performance of ultrasonography in predicting endometrial malignancy in women with postmenopausal vaginal bleeding often excluded

individuals using HRT or when included in the analysis, the overall numbers were too small to draw any significant conclusions regarding the outcome of investigation.[15–17]

The aim of this study is to estimate the risk of endometrial cancer in postmenopausal women presenting with vaginal bleeding using estrogen–progestogen HRT regimens. In addition, we aim to assess if the duration of HRT use has an effect on the risk of diagnosing endometrial cancer.

Methods

This study of consecutive postmenopausal women presenting with vaginal bleeding was conducted at a gynaecological oncology centre in the UK, between February 2006 and April 2011. Women were diagnosed postmenopausal after at least 12 months of amenorrhoea. Excluded from the study were premenopausal women, asymptomatic women with an incidental finding of increased endometrial thickness on imaging, asymptomatic women with abnormal endometrial cytology found on cervical smear and women with a history of hysterectomy.

All women underwent transvaginal ultrasound scanning as the initial investigation tool to evaluate the endometrium. We used greyscale ultrasonography to measure the double-wall endometrial thickness in an anteroposterior dimension, in the sagittal plane from one basalis layer to the other. In keeping with departmental guidelines, when the endometrial thickness measured less than 5 mm no further investigations were performed as evidence suggests a low probability of cancer below this threshold.

Women found to have an endometrial thickness equal to or greater than 5 mm had endometrial sampling performed using an endometrial Pipelle device (Pipelle de Cornier; Laboratoire CCD, Paris, France). Endometrial biopsy was also performed in cases where the endometrial thickness was not clearly visualized on transvaginal ultrasonography. Hysteroscopic evaluation of the endometrium with biopsy was performed if Pipelle biopsy was not possible or did not yield sufficient tissue for histological diagnosis. In spite of benign histology on Pipelle® biopsy, hysteroscopy was also performed in cases where endometrial thickness was greater than 10 mm, due to possibility of an endometrial polyp.

Data regarding the following characteristics were recorded for all women: age at presentation, body mass index (BMI) calculated as weight (kg)/(height [m])^2, use of HRT, presence of hypertension and diabetes, previous history of breast cancer, use of tamoxifen at presentation, amount of blood lost and frequency of the episodes of vaginal bleeding. The above characteristics, endometrial thickness measurement and the histology results were collected and recorded prospectively in an electronic database.

Ethical approval for the use of the postmenopausal clinic database was granted by the National Research Ethics Service Committee South Central – Oxford C on 29 July 2011 (reference number: 11/SC/0285). The local Research and Development study number is 2011O&G06L (120-08-11).

The distributions of continuous variables were not symmetric. To test for normality, the Shapiro–Wilk *W* test was used, as was the *q–q* plot to investigate normality graphically (results not shown). There was no evidence to suggest that data were normally distributed, hence in the descriptive statistics for continuous variables, we report median and interquartile range. To test any differences we used a non-parametric Wilcoxon rank sum (Mann-Whitney) test. Chi-squared test was used after checking the expected assumptions. An adjusted logistic regression was carried out to investigate the odds of HRT controlling for clinical characteristics and cancer diagnosis. All analyses were done using STATA software, version 11.2 SE (Stata Corporation, College Station, TX, USA).

Results

Over a 62-month period, 4847 women were investigated for postmenopausal vaginal bleeding. The majority of women (4097, 84.5%) did not use any HRT preparation at the time of initial referral and 750 (15.5%) women were using combined HRT preparations. Of the women using HRT preparations, 194 (25.8%) were unable to provide details about the duration of HRT use. Two women using estrogen-only HRT preparations were excluded from the analysis.

The median age and BMI of women using HRT were significantly lower than the values for women not using HRT preparations ($P < 0.0001$). The differences on the above characteristics, although statistically significant, have no clinical relevance. In addition, a smaller percentage of women using HRT compared with women not using any HRT preparations were previously diagnosed with diabetes or hypertension as shown in Table 1.

A total of 298 (6.1%) women were diagnosed with endometrial carcinoma during the study period. Type I endometrial cancer accounted for 87.9% of all the cases. As shown in Table 2, women using HRT preparations were significantly less likely to be diagnosed with type I endometrial cancer ($P < 0.0001$). Significant difference was also observed in the incidence of type II endometrial cancer cases diagnosed in women using HRT compared with women not using HRT. However, only one case of type II endometrial cancer was diagnosed in women using HRT preparations, which makes it difficult to draw firm conclusions. The overall risk of diagnosing endometrial cancer in women using HRT preparations was significantly lower compared with women not using HRT.

On a multivariate analysis shown in Table 3, when adjusting for other clinical characteristics such as age, BMI, bleeding patterns, diabetes and hypertension, women using HRT were less likely to be diagnosed with endometrial cancer (odds ratio = 0.229, 95% CI 0.116–0.452; $P < 0.0001$).

The risk of diagnosing endometrial cancer (per 1000 women) in relation to duration of HRT use is graphically presented in Figure 1. Due to small number of endometrial cancer cases in women using HRT, three-year intervals were used. The risk of diagnosing endometrial cancer in women presenting with postmenopausal vaginal bleeding appears to increase with longer duration of HRT use, but this is not statistically significant

Table 1 Clinical characteristics of the individuals in the study

Characteristics	HRT use *N* = 750	No HRT *N* = 4097	*P* value
Age (years)	58 (54, 62)	59 (54, 68)	<0.0001*
BMI†	27 (24, 30)	28 (25, 33)	<0.0001*
Diabetes	24 (3.28%, 2.1–4.7%)	260 (6.3%, 5.6–7.1%)	0.001‡
Hypertension	154 (21%, 18–24%)	1109 (27%, 26–28%)	<0.0001‡
Frequency of bleeding†			
Single episode	422 (57%, 53–60%)	2022 (50%, 48–51%)	<0.0001‡
Recurrent	323 (43%, 40–47%)	2062 (50%, 49–52%)	
Amount of bleeding†			
Spotting	110 (15%, 12–18%)	718 (18%, 17–19%)	0.117‡
Light	471 (64%, 60–67%)	2495 (62%, 61–64%)	
Heavy	159 (21%, 19–25%)	798 (20%, 19–21%)	
Endometrial thickness	5.2 (3.5, 7.9)	4.6 (2.90, 8.8)	0.0024*

HRT, hormone replacement therapy
Values are median (interquartile range), number (percentage, 95% CI)
*Two-sample Wilcoxon rank sum test (Mann-Whitney test)
†Percentages or medians worked on less numbers from the overall due to missing values
‡Chi-squared test

Table 2 Diagnosis of endometrial cancer in the group of women using and those not using HRT preparations at the time of referral

Endometrial cancer	HRT use (*N* = 750)	No HRT (*N* = 4097)	*P* value
Type I	9 (1.2%, 0.6–2.3%)	253 (6.2%, 6.5–9.6%)	<0.0001*
Type II	1 (0.13%, 0.003–0.7%)	35 (0.9%, 0.6–1.2%)	0.035*

*Chi-squared test

($P = 0.420$). The risk of diagnosing endometrial cancer was higher for women who were unable to provide information about the duration of HRT use. However, the risk was not significantly different even when compared with women using HRT for 1–3 years.

Table 3 The effect of using HRT on the odds ratio for endometrial cancer adjusted for age, BMI, bleeding patterns, hypertension and diabetes

Variables	Odds ratio	*P* value	95% CI
HRT			
No	1	1	1
Yes	0.229	<0.0001	(0.116, 0.452)
Age	1.052	<0.0001	(1.039, 1.065)
BMI	1.050	<0.0001	(1.034, 1.066)
Bleeding frequency			
Single	1	1	1
Recurrent	5.310	<0.0001	(3.847, 7.332)
Hypertension			
No	1	1	1
Yes	1.368	0.0220	(1.046, 1.789)
Diabetes			
No	1	1	1
Yes	1.486	0.049	(1.001, 2.204)
Amount of bleeding			
Spotting	1	1	1
Light	0.890	0.473	(0.647, 1.223)
Heavy	1.119	0.589	(0.743, 1.685)

As shown in Table 4, the majority of women (49%) using HRT preparations were found to have normal endometrial histology. In 40% of women using HRT, the endometrial thickness measured less than 5 mm and the symptoms in this group of women were attributed to genital tract atrophy. The incidence of benign endometrial polyps in women using HRT was significantly lower than in women not using HRT ($P < 0.0001$).

Discussion

This study shows that the likelihood of diagnosing endometrial cancer in postmenopausal women when presenting abnormal vaginal bleeding and using combined HRT preparations is significantly lower when compared with women not using HRT. In this study, we did not distinguish between women using continuous combined and cyclical HRT preparations. However, the majority of women in the study used continuous

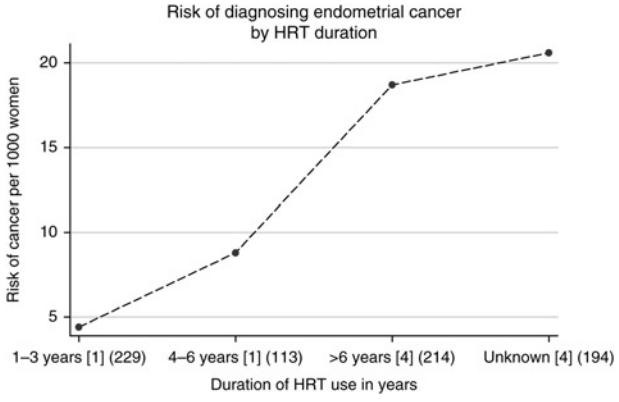

Figure 1 The risk of diagnosing endometrial cancer in relation to duration of HRT use (3-year intervals: 1–3 years, 4–6 years, >6 years and unknown duration). [#]=number of individuals with cancer; (#)=total number of women using HRT in each category

Table 4 Outcome of investigation in women using and those not using HRT preparations at the time of referral

Outcome of investigations	HRT use *N* (%, 95% CI)	No HRT *N* (%, 95% CI)	*P* value
Atrophy (ET <5 mm)	300 (40.0%, 36.4–43.6)	1920 (46.8%, 45.3–48.4)	=0.0001*
Normal histology	370 (49%, 46–53%)	1316 (32%, 31–34%)	<0.0001*
Endometrial polyps	51 (6.8%, 5.1–8.8%)	472 (12%, 11–13%)	<0.0001*
Endometrial hyperplasia	15 (2.0%, 1.1–3.3%)	76 (1.9%, 1.5–2.3%)	0.788*
Endometrial cancer	10 (1.3%, 0.6–2.4)	288 (7.0%, 6.3–7.8)	<0.0001*
Other	4 (0.5%, 0.1–1.3)	25 (0.6%, 0.4–0.9)	0.802

Overall *P* value <0.0001*
*Chi-squared test

combined preparations and the endometrial atrophy induced by the daily administration of progestogens in this group is likely to account for the lower incidence of cancer in the overall population of women using HRT. Similarly, a lower incidence of diagnosing endometrial polyps was observed in women using HRT. This is likely to be related to the antiproliferative effect of the progestogens in the endometrium.[18]

In our study, we used transvaginal ultrasonography to select the patients who require endometrial biopsy. Lin *et al.*[19] showed that the mean endometrial thickness measurement in asymptomatic postmenopausal women was 5.3 mm in the group using continuous combined HRT and 6.6 mm in the group using cyclical combined preparations. Similarly, Levine *et al.*[20] found that endometrial thickness measurement was greater in the group of postmenopausal women using cyclical compared with continuous HRT (8.3 versus 6.2 mm).Both these studies did not report any cases of endometrial cancer among women using combined HRT preparations.[19,20] In a study of 327 postmenopausal women (including 46 women with abnormal vaginal bleeding) using estrogen only or combined HRT preparations, Holbert *et al.*[21] reported one case of endometrial cancer in a patient with abnormal vaginal bleeding using estrogen-only HRT.

Langer *et al.*[22] reported a 99% negative predictive value of transvaginal ultrasonography and use of an endometrial thickness threshold of 5 mm for detecting serious endometrial pathology in asymptomatic postmenopausal women using estrogen-only or combined HRT preparations. However, no cases of endometrial cancer were found in women with endometrial thickness measurement of less than 5 mm, including cases in women using estrogen-only preparations. Langer *et al.* reported no cases of endometrial cancer in women using combined HRT preparations.

Our study did not distinguish between women using continuous and cyclical HRT preparations. In addition, we investigated all women in the same way, using an endometrial thickness of 5 mm on transvaginal ultrasonography as a threshold to perform Pipelle biopsy. This practice would be in line with the results of the studies mentioned previously.[19–22] However, the majority of the postmenopausal women included in these studies were asymptomatic and endometrial thickness thresholds for women with vaginal bleeding using HRT are not well known.

It is common practice for the primary care practitioners in the UK to stop HRT in women presenting with postmenopausal bleeding prior to referring them to secondary care for further investigation. Although there is no evidence to support this practice, it leads to increased anxiety in women and is likely to enhance the negative opinion about HRT among patients. Based on the findings of our study, we suggest that it is not necessary to advise women to stop combined HRT preparations prior to investigation of abnormal vaginal bleeding. If transvaginal ultrasonography is used as the initial tool to investigate women with postmenopausal vaginal bleeding, it is likely that women using cyclical combined preparations will require further evaluation by endometrial biopsy due to variation in endometrial thickness during the hormonal cycle.

In conclusion, we report significantly lower incidence of endometrial cancer in symptomatic postmenopausal women using combined HRT preparations when compared with women not using HRT. Despite the inherent limitations with observational research and the bias generated by confounding factors, the results of this large study can be used to guide clinicians when investigating women with postmenopausal vaginal bleeding. Women using combined HRT preparations can be investigated on a less urgent basis depending on the available resources.

Competing interests: None declared.

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To whom it may concern,

I confirm that Dr Nikolaos Burbos had the following contributions to the above manuscript:

1. He conceived the idea for the study.
2. He designed and database for the data collection and collected more than 75% of the data included in the study. He also had an oversight of the data collected by his colleagues.
3. A medical statistician carried out the analysis of the data. Dr Burbos arranged study group meetings to plan the analysis, review the results, interpret and present the clinical significance of the findings.
4. He wrote the manuscript, incorporated changes from his co-authors and submitted the manuscript for publication. He responded to the comments made by the reviewers and submitted the revised version of the manuscript.

Yours sincerely,

Joaquin J. Nieto

Chapter 8. Management of postmenopausal women with vaginal bleeding when the endometrium cannot be visualised.

8.1 Introduction

A number of patients with postmenopausal vaginal bleeding (PMB) that undergo transvaginal ultrasonography (TVUS), will not have an adequate assessment of the endometrium. There are no data in the literature to provide an estimation of the endometrial cancer risk in this group of patients. In this chapter, I present a study conducted to determine the risk of endometrial cancer in women with PMB that have an unsatisfactory TVUS examination of the endometrium.

8.2 Literature review

In clinical practice, TVUS examination of the endometrium in women with PMB is not always possible. Presence of uterine fibroids, axial uterus or previous surgery may not allow adequate views of the entire length endometrium [214].

Most studies evaluating the role of TVUS in women with PMB did not report the rate of patients that had an unsatisfactory examination of the endometrium [123, 153, 215, 216]. In a large study of 752 women investigated for PMB, an endometrial thickness measurement was feasible for all patients [194]. In a different study, 213 postmenopausal women had TVUS to assess the endometrial thickness and none of the patients had suboptimal visualisation of the endometrium [217]. It is not clear, however, if the authors of the above studies excluded from the analysis cases where endometrial thickness could not be visualised on TVUS.

There is significant variation in the rates of inadequate examination of the endometrium on TVUS in women with PMB. The authors of a large study of 1168 women investigated for PMB, found that endometrial thickness could not be assessed in 30 (2.8%) patients [108]. Among them, one patient was diagnosed with endometrial cancer and one with atypical hyperplasia of the endometrium. In a different study, endometrial thickness was not

visualised in 23 (5.2%) of 442 women with PMB [189]. Of the 23 patients, endometrial biopsies revealed 4 cases of hyperplasia. The authors of another study reported that endometrial thickness was not clearly identified in 5 (2.7%) of 182 women with PMB [218]. One patient with unsatisfactory TVUS examination was diagnosed with endometrial cancer. Cameron et al reported that 7 (18.4%) of 38 patients in their study had an unsatisfactory TVUS assessment, but none of them was found to have endometrial cancer [219]. Epstein et al found one case of endometrial cancer among 8 patients with unsatisfactory TVUS, in their study of 107 women with PMB [99]. Bronz et al found the rate of inadequate assessment of the endometrium was 1.7% and no cases of cancer were diagnosed in this subgroup of patients [220]. Other studies found that the rate of unsatisfactory examination of the endometrium is higher in patients diagnosed with endometrial cancer compared to patients found to have a benign pathology (33% versus 8%, respectively) [221]. Similar findings about the rates of inadequate examination of the endometrium using TVUS are reported by other authors, but the incidence of endometrial cancer in this subgroup of patients was not presented [112, 113, 222].

The expertise of the operator performing the TVUS, may impact on the rate of inadequate examinations. In a study of 752 women with PMB, where TVUS examinations were performed by two physicians all patients had an adequate assessment of the endometrium [194]. In another study, 213 consecutive patients had a satisfactory assessment of the endometrium, performed by a single operator [217]. However, Garuti et al reported that although two experienced ultrasonographers performed all the examinations in their study, in 5.2% of the patients measurement of endometrial thickness was not possible [189]. Often, the experience or number of operators performing the investigations is not reported [219, 222]. Interestingly, studies assessing operator's experience in obtaining a reproducible endometrial

thickness measurement on TVUS show acceptable intraobserver and interobserver variation [155, 223, 224].

8.3 What does this study add?

This is the first study to estimate the risk of endometrial cancer in women with PMB that have an unsatisfactory TVUS assessment of the endometrium. The results of the study can be used to counsel patients regarding further investigations. In addition, the results of the study can be useful for developing diagnostic pathways for women with PMB.

8.4 What went well?

I conceived the idea for the study while reviewing data from the literature on the role of TVUS in investigating women with PMB. I observed that studies often excluded from analysis a small number of patients that had inadequate assessment of the endometrium. I was interested in estimating the incidence of the problem in clinical practice, and also to determine the risk of endometrial cancer in this subgroup of patients with PMB. I presented the idea to my co-authors and discussed the data analysis and presentation with the statistician. I wrote and submitted the manuscript for publication.

During this phase of my research, I realised the importance of carefully reviewing the methodology of the research studies. As most of the studies in a particular subject follow the same methodology, often there are no data on the outcomes or management for certain subgroups of patients. Frequently, this has implications for clinical practice but also generates more questions and ideas for future research projects.

8.5 What could have been done differently?

In this study we did not investigate the causes for unsatisfactory assessment of the endometrium on TVUS. Often, reasons such as presence of fibroids or obesity were recorded but the data were incomplete. In addition, we did not assess the rate of inadequate

examinations and proportion of patients diagnosed with endometrial cancer for individual operators.

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AOGS MAIN RESEARCH ARTICLE

Management of postmenopausal women with vaginal bleeding when the endometrium can not be visualized

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Key words
Postmenopausal bleeding; endometrium; uterus; endometrial thickness; transvaginal ultrasound; endometrial cancer

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Conflict of interest
The authors have stated explicitly that there are no conflicts of interest in connection with this article The data presented in this manuscript are part of an ongoing project for evaluation and improvement of diagnostic pathways for women with postmenopausal vaginal bleeding.

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Abstract

Objective. To determine the risk of endometrial cancer when endometrial thickness is not visualized using ultrasonography. *Design*. Cross-sectional study. *Setting*. Gynecological oncology center in the United Kingdom. *Population*. All postmenopausal women referred with vaginal bleeding. *Methods*. All women were investigated using gray-scale transvaginal ultrasonography. Women were arbitrarily stratified into four groups according to the endometrial thickness measurement. Women with endometrial thickness that was not adequately visualized on ultrasonography were included in a separate group. *Main outcome measures*. Endometrial cancer diagnosis. *Results*. Over a 50-month period, 4454 women were investigated for postmenopausal vaginal bleeding. A total of 259 (6%) of women were diagnosed with endometrial carcinoma. Endometrial thickness measured 5–9.9mm in 1201 (27%), 10–14.9mm in 468 (11%), 15–19.9mm in 209 (5%), and equal to or greater than 20mm in 197 (4%) of women. In 174 (4%) of women, the endometrial thickness was not visualized on transvaginal ultrasonography. For women where the endometrial thickness was not adequately visualized, the final histology included benign endometrium (124), endometrial cancer (26), endometrial polyps (11), endometritis (7), and other pathology (7). The odds of endometrial cancer in women where the endometrial thickness was not visualized were found to be significantly higher than the odds of cancer for women with an endometrial thickness of 5–9.9mm (OR = 5.23, 95%CI 3.10–8.85, p-value <0.0001). *Conclusions*. For women presenting with postmenopausal bleeding and where the endometrial thickness cannot be adequately visualized on ultrasonography, hysteroscopic evaluation is recommended.

Abbreviations: HRT, hormone replacement therapy; NPV, negative predictive value; PMB, postmenopausal bleeding; PPV, positive predictive value; TVS, transvaginal ultrasonography/ultrasound.

Introduction

Postmenopausal vaginal bleeding (PMB) is a common clinical problem. In the majority of cases the bleeding is due to benign conditions such as genital tract atrophy or endometrial polyps. The aim of investigation in women presenting with PMB is to exclude endometrial carcinoma. More than 75% of cases of endometrial cancer present in postmenopausal women and vaginal bleeding is the main presenting

Key Message

There is a 15% risk of endometrial malignancy in women presenting postmenopausal bleeding when the endometrium is not adequately seen with transvaginal ultrasound and hysteroscopic evaluation; endometrial biopsies are recommended for these women.

symptom (1,2). In the UK, according to national recommendations, all women presenting with postmenopausal vaginal bleeding should be referred for investigation in order to exclude malignancy (3). Either endometrial biopsy or transvaginal ultrasonography (TVS) can be used as the initial tool to evaluate the endometrium in symptomatic postmenopausal women (4). Women with an endometrial thickness measuring less than 4–5mm on transvaginal ultrasonography have a low risk of endometrial pathology (5,6). A thicker lining should be evaluated by an office-based endometrial sampling device or hysteroscopy and directed biopsy. Available evidence suggests that in 2.8–10% of cases the endometrium cannot be adequately visualized on TVS (5,7). However the risk of endometrial cancer in this group of women has not been studied previously.

The objective of this study was to determine the risk of endometrial cancer in cases where endometrial thickness cannot be visualized using TVS. In addition, we calculated the performance of ultrasonography in the prediction of malignancy according to endometrial thickness measurement.

Material and methods

This prospective study was conducted over a 50-month period in a gynecological oncology center in the UK. All postmenopausal women presenting with vaginal bleeding during this period were included in the study. We excluded premenopausal women, asymptomatic women with an incidental finding of increased endometrial thickness on imaging, asymptomatic women with abnormal endometrial cytology found in a cervical smear test, and women with a history of hysterectomy.

All women underwent TVS as the initial investigation tool to evaluate the endometrium. The procedures were performed by gynecologists trained in the use of ultrasonography for the investigation of women with PMB. In our unit we use gray-scale ultrasound and measure the double-wall endometrial thickness in an anteroposterior dimension from one basalis layer to the other. In cases where the endometrial thickness measured <5mm, no further investigations were performed. Women found to have an endometrial thickness ≥5mm had endometrial sampling performed using an endometrial Pipelle® device. The unit protocol for the management of women in whom the endometrial thickness was not clearly visualized was to perform endometrial sampling by Pipelle® device or hysteroscopic evaluation of the endometrium and endometrial biopsy if outpatient biopsy was not possible or did not yield sufficient tissue for histological diagnosis.

If a patient was investigated by either ultrasonography or endometrial biopsy and cancer was previously excluded, then these women were eligible for inclusion. Hence, some women were included in the study more than once.

Ethical approval for the use of the postmenopausal clinic database was granted by the National Research Ethics Service Committee South Central–Oxford C on 29 July 2011 (reference number: 11/SC/0285). The local Research and Development study number is 2011O&G06L (120–08–11).

Statistical analysis

For analysis, women were arbitrarily stratified in four groups according to the endometrial thickness measurement: 5–9.9mm, 10–14.9mm, 15–19.9mm and ≥ 20mm. Women with an endometrial thickness that was not adequately visualized on ultrasonography were included in a separate group. We excluded from the final analysis women found to have endometrial thickness measuring <5mm on TVS. We included in the analysis both type I (endometrioid histology) and type II (non-endometrioid) endometrial cancers diagnosed during the study period. For women with atypical hyperplasia diagnosed on Pipelle® biopsy, hysterectomy was recommended, as women in the study were postmenopausal. Cases showing no malignancy in the final histology (hysterectomy specimen) were included in the non-cancer group. If malignancy was diagnosed following hysterectomy, women were included in the cancer group. For women who did not undergo hysterectomy, hysteroscopic endometrial evaluation was performed and the result of the biopsies obtained was used as the histological outcome for the statistical analysis.

There was no evidence to suggest that continuous variables were normally distributed. Hence, in the descriptive statistics for continuous variables, we report median and interquartile range. To avoid inflating the type I error rate, loss of power, residual confounding and bias, continuous predictor variables were not categorized (8–10).

We calculated the sensitivity, specificity, positive predictive values (PPV) and negative predictive value (NPV) for each endometrial thickness group. Logistic regression analysis was used to estimate the risk of cancer for each endometrial group. The reference group was the one including women where endometrial thickness was not visualized on ultrasonography. All analyses were done using STATA software, version 11.1 SE (Stata Corporation, College Station, TX, USA).

Results

During the study period, 4454 women were investigated for PMB. A total of 259 (6%) women were diagnosed with endometrial carcinoma. The remaining 4195 (94%) were included in the non-cancer group for analysis. Overall, 2205 (49%) women were found to have an endometrial thickness measuring <5mm on transvaginal ultrasound. In 174 (4%) women, endometrial thickness was not visualized. For women where endometrial thickness was not adequately visualized, the final histology included benign endometrium

Table 1. Clinical characteristics of patients in each group according to endometrial thickness measurements (median and interquartile range for continuous values and numbers and percentages for categorical values).

Clinical characteristics	Endometrial thickness measurement				
	5–9mm	10–14.9mm	15–19.9mm	≥20mm	Not visualized
Age (years)	57 (53, 64)	60 (55, 68)	64 (57, 70)	64 (58, 73)	63 (57, 71)
BMI (kg/m^2)	29 (25, 34)	31 (21, 37)	32 (27, 38)	32 (27, 39)	28 (24, 34)
Duration of HRT (years)	5 (2, 10)	5 (2, 9)	7 (3, 11)	4 (3,6)	6 (2, 10)
Patients using HRT, *n* (%)	281 (23%)	64 (14%)	13 (6%)	14 (7%)	17 (10%)
Tamoxifen use (years)	3 (1, 5)	2 (1, 4)	5 (2, 5)	4 (2, 5)	5 (2, 5)
Patients using tamoxifen, *n* (%)	29 (2%)	31 (7%)	19 (9%)	33 (17%)	10 (6%)
Endometrial cancer					
No	1162 (97%)	395 (84%)	160 (77%)	129 (65%)	148 (85%)
Yes	39 (3%)	73 (17%)	49 (23%)	68 (34%)	26 (15%)
No. of patients (%)	1201 (27%)	468 (11%)	209 (5%)	197 (4%)	174 (4%)

HRT, hormone replacement therapy.

($n = 124$), endometrial cancer ($n = 26$), endometrial polyps ($n = 11$), endometritis ($n = 7$) and other pathology ($n = 7$).

We found no significant difference in the mean endometrial thickness between women with type I and type II endometrial cancers. In addition, no significant difference was observed in the percentage of women with inadequately visualized endometrium between the above groups.

Table 1 shows the number of women in each group as stratified by endometrial thickness measurement and their clinical characteristics.

Figure 1 shows the risk of a woman being diagnosed with endometrial cancer in relation to endometrial thickness measurement, calculated by logistic regression analysis. The group of women where the endometrium was not adequately visualized was used as reference category. For women on hormone therapy and those using tamoxifen for treatment of breast cancer there was not an acceptable endometrial thickness cut-off measurement that could be used to exclude endometrial pathology. Figure 2 shows the odds of cancer excluding women using hormone replacement therapy (HRT) and those using tamoxifen.

Table 2 shows the specificity, sensitivity, PPV, NPV and the odds ratios for each endometrial thickness measurement category. We observed an increase in the positive predictive value for cancer diagnosis with increasing endometrial thickness.

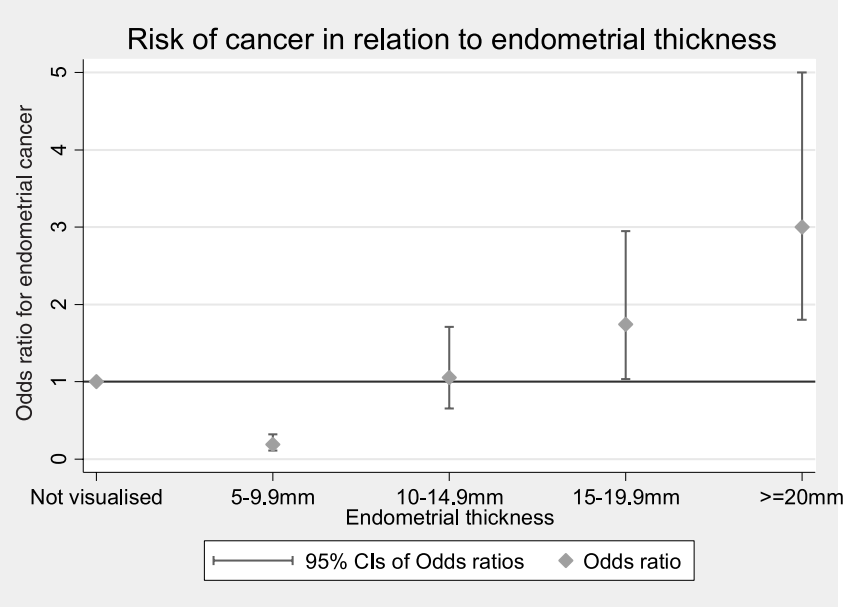

Figure 1. Odds ratio for endometrial cancer in relation to endometrial thickness measurements.

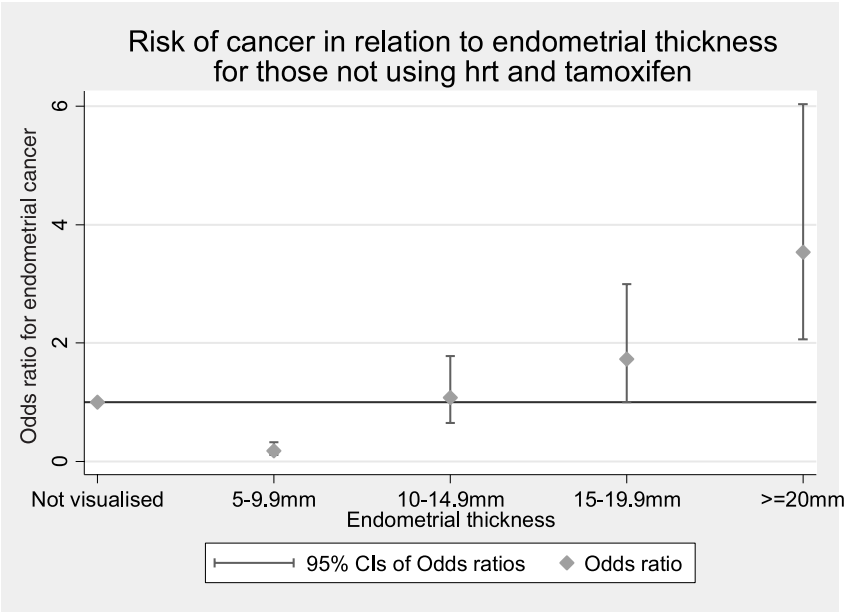

Figure 2. Odds ratio for endometrial cancer in relation to endometrial thickness measurements for women not using hormone replacement therapy (HRT) preparations and tamoxifen, CI, confidence interval.

Table 2. Performance of endometrial thickness measurements in predicting endometrial cancer.

Endometrial thickness	Performance characteristics			
	Sensitivity	Specificity	PPV	NPV
5–9.9mm	15.1% (10.9–20.0)	72.3% (70.9–73.7)	3.2% (2.3–4.4)	93.2% (92.3–94.1)
10–14. 9mm	28.2% (22.8–34.1)	90.6% (89.7–91.5)	15.6% (12.4–19.2)	95.3% (94.6–96.0)
15–19.9mm	18.9% (14.3–24.2)	96.2% (95.6–96.7)	23.4% (17.9–29.8)	95.1% (94.4–95.7)
≥20mm	26.3% (21.0–32.1)	96.9% (96.4–97.4)	34.5% (27.9–41.6)	95.5% (94.8–96.1)
Not visualized	10.0% (6.6–14.4)	96.5% (95.9–97.0)	14.9% (10.0–21.1)	94.6% (93.8–95.2)

PPV, positive predictive value, NPV, negative predictive value.

Discussion

The principal aim of investigation in women with PMB is to exclude malignancy. TVS and/or office-based endometrial biopsy is commonly used as the initial tool for the evaluation of the endometrium. TVS is used to distinguish women who require tissue sampling to exclude endometrial cancer. The risk of endometrial malignancy in women with PMB and endometrial thickness ≤4mm is around 1:917 and hence endometrial biopsy is not indicated (11). Women found to have an ultrasound endometrial thickness ≥5mm are evaluated further using office-based biopsy or hysteroscopy. A meta-analysis of 35 studies assessing the accuracy of TVS for investigating women with PMB described a sensitivity of 96% and a specificity of 53% for endometrial malignancy using an endometrial thickness threshold of ≥5mm. The same authors reported that with an endometrial thickness threshold of 5mm the sensitivity remained unchanged but specificity improved to 61% (6). More recent data suggest that an endometrial thickness cut-off measurement of 3mm should be used as the threshold for excluding malignancy in women with PMB (12). However, for women using hormone therapy there is no standard threshold for determining the need for further endometrial assessment. Endometrial thickness measurement can vary from 2 to 15mm in women using hormone therapy. No cases of endometrial cancer were found in women using estrogen and progestin HRT preparations (13). Similarly, the value of TVS in endometrial evaluation is limited in women using tamoxifen for management of breast cancer. The majority of endometrial pathology diagnosed in this group of women represents benign polyps (14).

Additional imaging techniques, such as saline contrast sonohysterography, evaluation of sonographic characteristics of the endometrium, power Doppler ultrasound, three-dimensional ultrasound measurement of endometrial volume and use of three-dimensional power Doppler angiography in discriminating between malignant and benign endometrial disease in women with postmenopausal vaginal bleeding, have also been studied (15–20). However, the results are conflicting and the value of these methods as

the initial step for investigation of women with PMB is uncertain.

Initial testing using ultrasonography appears to be more cost-effective in the investigation of PMB, assuming an endometrial cancer prevalence of 5%. When the prevalence of endometrial disease is higher, for example 10%, then the use of endometrial biopsy as the initial test is more cost-effective (21). Pipelle® biopsy has been shown to perform better than any other endometrial sampling devices, with a reported detection rate of 99.6% for endometrial carcinoma in postmenopausal women (22). Clark et al. (23) found that the post-test probability of endometrial cancer in postmenopausal women after a negative sampling result using Pipelle® was 0.8% (95%CI 0.2–3.1%).

In order to be considered 'normal', a thin endometrial lining on transvaginal ultrasonography must also be regular and clearly visible over the totality of the uterine cavity (24). Occasionally it is not possible to measure endometrial thickness using transvaginal ultrasound. Often this is due to the presence of uterine fibroids obscuring the view, lack of contrast with the surrounding myometrium or endometrial pathology. In 1.5–10.4% of women presenting with postmenopausal vaginal bleeding a reliable measurement of the endometrial thickness cannot be obtained. The reported prevalence of endometrial cancer in this group of women varies from 0 to 12.5% (5,25–28). The variable prevalence of malignancy is likely to reflect differences in the clinical characteristics of the women investigated and the sample size of the studies. In our study, more than 41% of women had a BMI of $\geq$30, compared with only 29% of women in the study by Van Doorn et al. (28).

In our study, the double-wall endometrial thickness was not adequately visualized on TVS in 4% of women presenting with PMB. Most commonly, the endometrium was not clearly visualized due to presence of uterine fibroids obscuring the view. The ultrasound scan investigations in our unit were performed by experienced examiners. Five main investigators performed the examinations during the study period. Therefore, it is unlikely that the experience of the operators had any significant impact on the outcome of the ultrasonographic examination. The majority of women with inadequately assessed endometrial thickness in this study had benign histology. However, we found that the risk of endometrial cancer in this group of women was 15%. This is significantly higher than the risk of malignancy reported by Karlsson et al. (5), but similar to the results reported by Van Doorn et al. (28).

In this manuscript, we did not report the outcome of investigation in women with endometrial thickness measuring less than 5mm. As we did not perform endometrial biopsy in this group of women we are unable to comment on the percentage of false-negative cases. We interrogated our database and found that five women who were found to have an endometrial thickness less than 5mm were subsequently diagnosed later with endometrial cancer. This represents a 0.0022% incidence of cancer amongst this group of women in our study population.

In conclusion, there was a 15% risk of diagnosing endometrial malignancy in women where endometrial thickness could not be adequately visualized on transvaginal ultrasound. This is clinically significant and should prompt endometrial sampling. If the test is negative, hysteroscopic evaluation of the endometrium should be offered to these women.

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To whom it may concern,

I confirm that Dr Nikolaos Burbos had the following contributions to the above manuscript:

1. He conceived the idea for the study.
2. He designed and database for the data collection and collected more than 75% of the data included in the study. He also had an oversight of the data collected by his colleagues.
3. A medical statistician carried out the analysis of the data. Dr Burbos arranged study group meetings to plan the analysis, review the results, interpret and present the clinical significance of the findings.
4. He wrote the manuscript, incorporated changes from his co-authors and submitted the manuscript for publication. He responded to the comments made by the reviewers and submitted the revised version of the manuscript.

Yours sincerely,

Joaquin J. Nieto

Chapter 9. Literature update and critical appraisal of the studies on predictive model development.

9.1 Relevant literature since publications

Several important studies concerning the investigation and management of women presenting with postmenopausal vaginal bleeding (PMB) have been published since the manuscripts included in this thesis were initially presented. The most relevant publications are summarised in this chapter.

In a multicentre randomised controlled trial, 200 women presenting with PMB who had an endometrial thickness of greater than 4 mm on ultrasonography and benign histology on biopsy, were randomised to hysteroscopy or expectant management [225]. The authors found no significant difference in the rate of recurrent bleeding within 12 months following initial randomisation, which was the primary objective of the trial, between patients that underwent hysteroscopy or expectant management (15.3% versus 18%, respectively; RR 0.85). Among patients that underwent hysteroscopy, there were 5 cases of atypical hyperplasia and 1 case of endometrial cancer detected within an endometrial polyp. Following hysterectomy, 3 cases of endometrial cancer and 3 cases of atypical hyperplasia were diagnosed on final histology. Hence, the authors conclude that 6% of cases of endometrial cancer and precancerous lesions are missed following a negative endometrial biopsy in women with PMB and an endometrial thickness >4 mm; further investigations should be considered in this group of patients.

Clarke et al conducted a systematic review including 40 790 women, to estimate the risk of endometrial cancer among patients presenting with PMB [226]. The authors estimated the prevalence of PMB in women with endometrial cancer was 91% (95% CI, 87%-93%) and the overall risk of endometrial cancer in women with PMB was 9% (95% CI, 8%-11%). Studies that excluded women using postmenopausal hormone therapy reported a significantly higher risk of endometrial cancer compared to studies including women using hormone therapy

(12% versus 7%, respectively). The risk of endometrial cancer was also higher in studies conducted in Western Europe (13%; 95% CI, 9%-19%) compared to studies of patients in Northern America (5%; 95% CI, 3%-11%) and Northern Europe (7%; 95% CI, 5%-8%). In addition, the authors found variations in the risk of endometrial cancer relating to the enrollment periods and types of the study (prospective or retrospective, cohort versus cross-sectional).

A recent systematic review and meta-analysis aimed to determine the optimal endometrial thickness threshold on ultrasonography to predict endometrial cancer in women with PMB, analysing data from 44 studies including 17 339 women [227]. The prevalence of endometrial cancer in 20 of the studies that evaluated the diagnostic performance of an endometrial thickness cut-off value equal to or greater than (≥) 5 mm, was 8.8%. At an endometrial cut-off value of 5 mm, the sensitivity and specificity of ultrasonography were 96.2% and 51.5%, respectively. The sensitivity of ultrasonography at an endometrial thickness cut-off of 5 mm was greater compared to that at a cut-off value of 4 mm (96.2% versus 95.7%, respectively). The authors believe this is likely to be due to heterogeneity between the studies assessing different endometrial thickness cut-off values. The negative predictive value at endometrial thickness cut-off values of 5 mm and 4 mm, was 99.3% and 99.4%, respectively. The risk of endometrial cancer in patients with an endometrial thickness measurement less than 5 mm was estimated to be 0.7%. The authors of this review conclude that an endometrial thickness threshold of 5 mm on ultrasonography has the best diagnostic accuracy for endometrial cancer in women investigated for PMB. The authors found only limited evidence on the role of other indices such as endometrial volume, vascularisation index and vascularity flow index on the assessment of patients with PMB.

Several new predictive models and clinical algorithms have been developed to estimate the risk of endometrial cancer in women with PMB. Giannella et al developed a diagnostic

predictive model using prospectively collected, self-reported data from 624 women investigated for PMB [228]. All patients had an endometrial thickness >4 mm on ultrasonography and underwent hysteroscopy. Women using postmenopausal hormone therapy that experienced irregular vaginal bleeding were also included in the study. A total of 15 predictor variables were collected and evaluated. Endometrial cancer was diagnosed in 72 (11.5%) of the patients. Recurrent vaginal bleeding (odds ratio [OR] = 2.96), presence of hypertension (OR = 2.01), endometrial thickness measurement (OR = 1.31) and the age of the patient (OR = 1.11) were identified by logistic regression as significant predictive variables and combined in the development of a diagnostic model, named RHEA. In a similar fashion to the predictive models included in this thesis [229, 230], a scoring system for each of the above variables was allocated. The diagnostic accuracy of the model, as estimated by the area under the ROC curve was 0.878 (95% CI, 0.842-0.908). In addition, the authors proposed a decision management algorithm for women with PMB based on the model developed. One of the main drawbacks of the RHEA model is its development was based on data from a highly selective group of patients with PMB, which is likely to affect its generalisability. A second concern with the model is the low number of endometrial cancer cases included compared to the number of potential predictors evaluated. This often leads to overfitting of the predictive model and potential poor performance in new datasets [231].

Dueholm et al performed a prospective study to develop models that predict the risk of endometrial cancer in patients with PMB, using a combination of clinical characteristics, gray-scale ultrasonographic findings, power doppler score and findings at gel infusion sonography (GIS) [232]. 174 patients found to have an endometrial thickness measurement ≥5 mm were included in the study. The sequence of the investigations performed varied depending on the referral pathway, with 104 patients undergoing transvaginal ultrasonography (TVUS) following an initial office-based endometrial biopsy. 72 (41%)

patients were diagnosed with endometrial cancer. Of the 4 predictive models presented, a model including endometrial morphology variables (endometrial thickness measurement, interrupted endomyometrial junction) and doppler score had an area under the ROC curve of 0.95 (95% CI, 0.92 – 0.99). There was no significant improvement in the diagnostic accuracy when irregular endometrial outline on GIS was included as a predictor to develop a new model, with an area under the ROC curve of 0.97 (95% CI, 0.94 – 0.99). The authors constructed a simple scoring system (REC score) based on 9 predictor variables. Each variable was given a score of 0 or 1 and the total score was calculated by adding the individual results. For a REC score ≥4, the sensitivity and specificity for endometrial cancer diagnosis were 91% and 94%, respectively.

Wong et al developed and internally validated two predictive models using retrospectively collected data from a large cohort of 4 383 women presenting with PMB [233]. All patients underwent a TVUS to measure the endometrial thickness and an office-based biopsy, regardless of the ultrasound scan findings. Hysteroscopy was performed if endometrial thickness measurement was ≥5 mm or in cases where endometrial assessment on TVUS was inadequate. 168 (3.8%) patients were diagnosed with endometrial cancer. The first model developed, named RAAMP, included the following predictor variables: frequency of vaginal bleeding (recurrent episodes), age at presentation, age at menopause, body mass index and nulliparity. A second model, named SPAR, included the value of the endometrial thickness measurement, nulliparity, age at presentation and recurrent episodes of bleeding as predictor variables. The authors defined recurrent bleeding as episodes of PMB separated by periods without bleeding. The diagnostic accuracy for the models developed, as described by the area under the ROC curve was 0.71 and 0.93 respectively ($p < 0.0001$). The authors found no difference in the diagnostic performance of the SPAR model compared to the use of TVUS alone. One of the main strengths of this study is the fact that all patients had a histological

evaluation of the endometrium. However, the prevalence of endometrial cancer in this cohort is low despite only a small percentage of patients reporting use of postmenopausal hormone therapy. It is likely the low prevalence of endometrial cancer is a result of the low body mass index in the population studied (median BMI 23.9, IQR 21.6-26.6).

Opolskiene et al had previously described the development of predictive models for women with PMB that incorporate TVUS characteristics of the endometrium [125]. Sladkevicius et al conducted a prospective study to validate the models developed at the same centre [234]. Women presenting with PMB and an endometrial thickness measurement ≥4.5 mm were included in the study. The validation cohort included 379 women, of which 93 (25%) were diagnosed with endometrial cancer. The authors found that the area under the ROC curve for endometrial thickness measurement alone was 0.79 (95% CI, 0.74 – 0.85) and had the worst diagnostic performance compared to the other models tested. A model combining endometrial thickness measurement, heterogenous echogenicity and the findings of areas of densely packed vessels had the best predictive ability, with an area under the ROC curve of 0.90 (95% CI, 0.86 – 0.94). The performance of all the models was slightly worse in the validation cohort compared to the development study. The authors concluded that the models can be used to individualise investigations in postmenopausal patients at high risk of endometrial cancer.

Dueholm et al attempted to optimise the diagnostic performance of the risk of endometrial cancer (REC) scoring system they had previously described [235]. The authors used data from the same cohort of patients in their initial study to develop two new predictive models [232]. These new models were adjusted for use in women found to have an endometrial thickness measurement ≥8 mm, as 92.5% of the cases of endometrial cancer and atypical hyperplasia were diagnosed in that group of patients. The first model included ultrasonographic findings of interrupted endomyometrial junction and the doppler score. The

second model used these variables plus the finding of irregular endometrial surface outline on gel infusion sonography [235]. The area under the ROC curve for the first model was 0.932 (95%CI, 0.89 – 0.98) and for the second model was 0.957 (95% CI, 0.92 – 0.99). The authors performed a prospective temporal validation study of 711 women, to assess the performance of the models developed. The prevalence of endometrial cancer in the validation cohort was 33.6%. In the validation group, the area under the ROC curve for the first model was 0.928 (95%CI, 0.90 – 0.95) and for the second model was 0.932 (95% CI, 0.90 – 0.97). The authors propose that although these models may not be applicable to all women presenting with PMB, they can be useful for identifying patients at high risk of endometrial cancer and atypical hyperplasia.

A recent study evaluated the diagnostic accuracy of urine and vaginal cytology for detection of endometrial cancer [236]. The study population included 103 women with suspected or diagnosed endometrial cancer and 113 women with PMB. The authors reported that combined urine and vaginal cytology had a sensitivity of 91.7% (95% CI, 84.9% - 96.2%) and a specificity of 88.8% (95% CI, 81.2% - 94.1%) for gynaecological cancer detection [236]. The authors concluded that the results need to be validated in prospective studies of women undergoing investigation for PMB.

9.2 Critical analysis of studies on predictive model development.

In this thesis I have described the development and internal validation of two diagnostic predictive models for women presenting with postmenopausal vaginal bleeding (PMB). Prior to commencing this work I reviewed the available literature and established the need for new predictive models in women with PMB. The predictive models available prior to these studies had several flaws related to the study design, sample size and predictor variable selection as described in previous chapters [152-158]. The critical analysis of the process for the development of the predictive models is based on the domains of the Checklist for critical

Appraisal and data extraction for systematic Reviews of prediction Modelling Studies (CHARMS) [237].

Population

The development of both predictive models was based on data from cross-sectional studies including all consecutive patients referred for investigation of PMB to secondary care. This is the preferred study design for derivation of diagnostic predictive models [238]. The prospective design of the study helps to minimise selection bias by correctly identifying patients and reducing the likelihood of missing information. The study population was clearly defined and the investigation pathway remained consistent during the study period. Cases with missing data were included in the analysis to avoid problems with generalisability of the model [239].

Sample size

One of the main strengths of this work was the adequate sample size. As a general rule, a minimum of 10 events (endometrial cancer cases) per each candidate predictor evaluated is recommended for model development [240-242]. The DEFAB and FAD31 models were developed based on study samples that included approximately 15 and 20 cases of endometrial cancer per predictor variable, respectively [229, 230]. Smaller sample sizes can lead to overfitting and poorer performance of a model [243].

Predictors

The potential predictors were selected and their definition agreed at the study design stage, based on data from the available literature. In addition to demographic characteristics and medical history, we investigated the value of including patient symptoms in the development of the model. The models developed as part of this thesis are of low complexity and include a small number of predictors. This is likely to help with the clinical use of the models. It has been shown that the presence of strong predictors in a model can improve its diagnostic

performance [243]. Both models presented in the thesis contained a predictor (frequency of bleeding) with an odds ratio of greater than 3.5.

It is also recommended that predictors evaluated at the stage of development should be clearly defined and reproducible in order to avoid problems with the generalisability of models [151, 244]. However, this may not be always possible, especially when clinical characteristics or symptoms are incorporated in predictive models [245]. I acknowledge that the main issue with the selected predictors included in the models relates to the definition of recurrent PMB. In addition to the expected variation between patients in recollection and/or reporting of symptoms, there is heterogeneity in the definition of recurrent PMB in the literature [228, 229, 233, 246-248]. Some of the studies used a similar definition to that used in the work included in this thesis [228, 233], while others defined recurrent PMB as bleeding that occurred after a previously evaluated episode [246-248]. We decided not to choose the latter definition for two main reasons. First, in clinical practice not all patients will present to their general practitioner immediately following an initial episode of PMB. It is likely that some patients will ignore the symptom and present only if the problem persists. This group of patients, with multiple episodes of bleeding prior to initial presentation, has a different risk of endometrial cancer compared to patients that experience only one episode of bleeding [228, 229, 233]. Second, for women with recurrent bleeding after an initial negative evaluation, the timing of subsequent investigations remains unclear. Clinician preferences or local guidelines regarding timing of investigations may contribute to the variation observed in the risk of endometrial cancer among women with recurrent PMB after a previously investigated episode [246-248].

Also, the definitions for the other predictors may not be widely acceptable. For example, because the risk of endometrial cancer decreases after treatment with tamoxifen is discontinued [249], we defined as current users only patients that received tamoxifen within 6

months from the time of referral for investigation of PMB. Other variables, such as the amount of vaginal bleeding, were subject to the patients' perception of the severity of their symptoms. Similarly, the definition of diabetes mellitus and hypertension is heterogenous and encompasses a wide spectrum of clinical disease depending on the timing of the disease onset, the degree of disease control and type of treatment used. It is uncertain, however, if the performance of the models will improve if each subgroup of these patients is considered separately or whether such an approach will lead to a more complicated model that is difficult to use in clinical practice.

Outcomes to be predicted

The models developed aimed to predict the probability of a binary outcome (benign pathology or endometrial cancer). The reference test for endometrial cancer diagnosis was histological confirmation. A benign pathology outcome was determined by either histological confirmation or an endometrial thickness measurement <5 mm on TVUS. The clinicians performing the TVUS were not blinded to candidate predictors, hence potential bias may exist in the reporting of the outcomes based on ultrasonography.

Several studies have previously combined patients diagnosed with endometrial hyperplasia and those with endometrial cancer in the same group, as the treatment for most of these patients is the same [153, 158]. However, we decided to include patients diagnosed with atypical endometrial hyperplasia in the same outcome group as patients with benign pathology because the aim of the models was to predict the risk of endometrial cancer and not conditions treated with hysterectomy. In addition, if all cases of endometrial hyperplasia were included in the same outcome group as endometrial cancer cases, this would lead to an overoptimistic predictive model.

The prevalence of endometrial cancer in the model development studies included in this thesis was lower than previously reported in the literature [116, 118]. Differences in the

prevalence of the outcomes between the derivation population and validation setting will impact on the performance of the predictive models [169]. In general, the value of a positive test result decreases as the prevalence of the condition decreases, even for tests with excellent sensitivity and specificity. Differences in the prevalence of the outcomes between the derivation and validation study will affect the calibration of the model in the validation sample [231, 250]. A simple approach to overcome this issue is to update the baseline risk of the original models to the patients in the validation sample [243, 250].

Internal validation

We tested the reproducibility (internal validation) of the predictive models in our development sample. Internal validation is aimed at evaluating the modeling process itself [169, 243]. It is needed to examine and correct the amount of overfitting in the development of predictive models [163, 231].

9.3 External validation

External validation is necessary to assess the generalisability/transportability of a predictive model [243, 251]. External validity is considered the stronger test for a model [252]. It represents the ability of the model to give valid predictions in populations that are different from but still related ('plausibly related') to the development population [253]. The predictive performance of models is usually decreased in a validation population compared to the development sample [254].

Several types of external validation methodologies have been reported in the literature [231]. Temporal validation refers to the evaluation of the predictive model on subsequent patients at the same centre [169, 253]. Geographical validation refers to the performance of the model tested at a different location [253]. In fully independent validation, the performance of the model is assessed by investigators not involved at the development stage, at other sites [243]. Domain validation refers to testing the model in very different patients than those from whom

it was derived [231, 255]. In methodological validation studies, the models are tested with data collected using alternative methods, while spectrum validation refers to testing in patients with different prevalence or severity of the outcome of interest [231, 243].

Factors that may affect external validation

Several factors may affect the model's performance in the validation cohort, including: deficiencies at the stage of model derivation, overoptimism of the predictions and differences in the case-mix between the development and validation settings [169, 231, 243, 255]. Case-mix describes the distribution of predictors (both included in the model and not) and outcomes [256]. In addition, if predictor variables used in the model are derived from an idiosyncratic population, validation in a different group of patients is likely to fail [257]. Another reason for poor validation is if one or more important predictors are missed at the stage of model development [231]. Several other characteristics such as ethnicity, a history of anovulation or family history of endometrial cancer can affect the risk of endometrial cancer [258-261]. These characteristics were not evaluated in the development studies for our predictive models. Data on these characteristics should be collected in the validation cohort and, if necessary, the predictive models can be updated using 'model extension' methods [243]. Further, the performance of a predictive model may change over time, and in our case can potentially be affected by changes in referral patterns for patients with PMB, prevalence of obesity and hysterectomy rates in the population.

Genetic factors may also affect the predictive performance of models. The risk of endometrial cancer in patients with Lynch syndrome is significantly higher than in the general population [262, 263]. Applying the models to a population of patients with a high pretest probability of the disease will result in high posttest probability of a positive result. In addition, as the sensitivity of the models is not 100%, the posttest probability of a negative result will also increase. Hence, a negative test will fail to correctly classify a higher

percentage of patients with endometrial cancer and will have a clinically significant impact. In addition, the pathogenesis of endometrial cancer in patients with Lynch syndrome is different compared to the general population [264] and many of these patients may not have traditional risk factors. Hence, DEFAB and FAD31 models may have significantly poorer performance in this particular group of patients. Genetic and epigenetic factors are often seen as an independent risk factor and can be incorporated in various prognostic models [265, 266]. However, it may not be possible to incorporate a history of Lynch syndrome as a variable during model development because most women with known Lynch syndrome have undergone prophylactic hysterectomy before menopause.

External validation study proposal

The aim of the proposed validation studies is to determine the performance of the two predictive models presented in this thesis on new data. I propose conducting two prospective validation studies: a. A multicentre cross-sectional study including all consecutive women referred with PMB, to undergo investigations in the secondary care and quantify the predictive performance of both models, b. A second validation study, conducted in the primary care setting, to test the performance of the FAD31 model (domain validation). The reason for the second study will be to determine if the FAD31 model can be used in primary care for risk stratification of patients with PMB (as shown in flowchart 3). Predictive models developed in secondary care are often found to have a decreased performance when validated in primary care populations [253, 256, 267]. The main reason for this is that changes in the setting can affect the case-mix of the predictors and outcomes [256]. Although national guidance recommends all women who present to primary care with PMB are referred to hospital for investigation, it is likely that some women are not referred - perhaps due to them declining the recommendation for referral, or being medically unfit for further investigations.

The population in both studies will include all consecutive women undergoing investigation for PMB (as previously defined). Women that have already undergone a hysterectomy, those who decline referral or investigations, and asymptomatic women found to have a thickened endometrium on TVUS will be excluded from the studies. Demographic and clinical data will be collected using an electronic database. In order to avoid subjective interpretation of the predictors by the clinician, predictors will be documented prior to the reference test (TVUS or endometrial biopsy result). In addition, the characteristics of the patients included in the validation cohort will be recorded to determine the degree of relatedness to the development cohort [231]. Data on the stage and histological grade of patients with endometrial cancer will also be collected. Cases with missing values will be included and imputation methods employed for handling of these data [268-270].

TVUS should be the first test to investigate women presenting with PMB. Women found to have an endometrial thickness measuring ≥4 mm on TVUS should undergo an endometrial biopsy [271]. An office-based endometrial sampling device can be used or, if this is not feasible, hysteroscopy and endometrial biopsy should be arranged. Women with findings suggestive of an endometrial polyp should undergo hysteroscopy to exclude focal pathology within the polyp. Reference standards to determine the outcomes will include: a. an endometrial thickness measurement <4 mm on TVUS (negative result); or b. histological diagnosis obtained either by hysterectomy (if performed), hysteroscopy or an office-based endometrial biopsy.

All reference tests will be performed under the two-week-wait rule as suggested by national recommendations. Under such circumstances, the interval between the initial presentation and any of the above reference tests will not affect any of the selected predictors or the outcomes (e.g., cancer will not grow de novo while the patient is waiting to undergo a hysteroscopy). Data on adverse effects of the reference tests including pain, heavy vaginal

bleeding, uterine infection or perforation will be recorded, as will the frequency of uninterpretable results (e.g., inadequate views of the endometrium on TVUS or insufficient endometrial biopsy samples). In cases of uninterpretable results, the decision of the treating clinician will be considered as the reference outcome.

The definition of recurrent PMB can include any of: a. bleeding episodes separated by periods without bleeding, or b. prolonged vaginal bleeding lasting more than 7 days. As mentioned above, patients diagnosed with atypical endometrial hyperplasia should be included in the same outcome group as patients with benign pathology.

All the investigations should be performed in dedicated clinics for management of women with PMB. The number of recruiting clinicians in the study will not be pre-specified as it will depend on arrangements in local settings. Definitions of the predictors will be available to clinicians (pop-up windows in the database) in order to standardise the data collection and to avoid interobserver variability. Expertise in TVUS and histopathology examination of the endometrial samples is already in place according to national standards. Although blinding of the readers of a test is important when assessing its diagnostic accuracy [272], it will not be possible for the clinician performing the TVUS to be blinded to the patient's clinical and demographic characteristics.

Regarding sample size, a minimum of 200 patients diagnosed with endometrial cancer will be required for the validation study. A smaller validation study containing between 100-200 cases of endometrial cancer may be adequate, but calibration performance of the models could be more reliably determined with more events [243, 273, 274].

A cross tabulation of the results of the predictive model's estimation by the results of the reference test will be presented. Variability between different subgroups of patients or between different recruiting centres will be evaluated and presented. The predictive performance of the models will be assessed by evaluating the overall performance,

calibration and discrimination [243]. The overall performance describes how close the model's predictions are to the actual outcomes and is commonly expressed using measures such as explained variation (R^2) or Brier score [243, 275]. The ability of the models to discriminate between patients with endometrial cancer and those without will be described by the area under the receiver operating characteristic curve [166]. The calibration of the model will be evaluated using graphical inspection (calibration plot), calibration in-the-large and calibration slope [243, 276]. In addition, decision curves for the predictive models will be constructed and the net benefit will be calculated in order to determine the clinical usefulness [243, 277, 278]. Recalibration or updating of the models using the new data may be required if the performance of the models in the validation sample is found to be inferior [256]. Updating of the predictive models will be preferable to developing new models as it avoids loss of the information generated in the derivation study [231, 255]. Further external validation of the updated predictive models will be considered. Results of the external validation will be reported according to published TRIPOD guidelines [279].

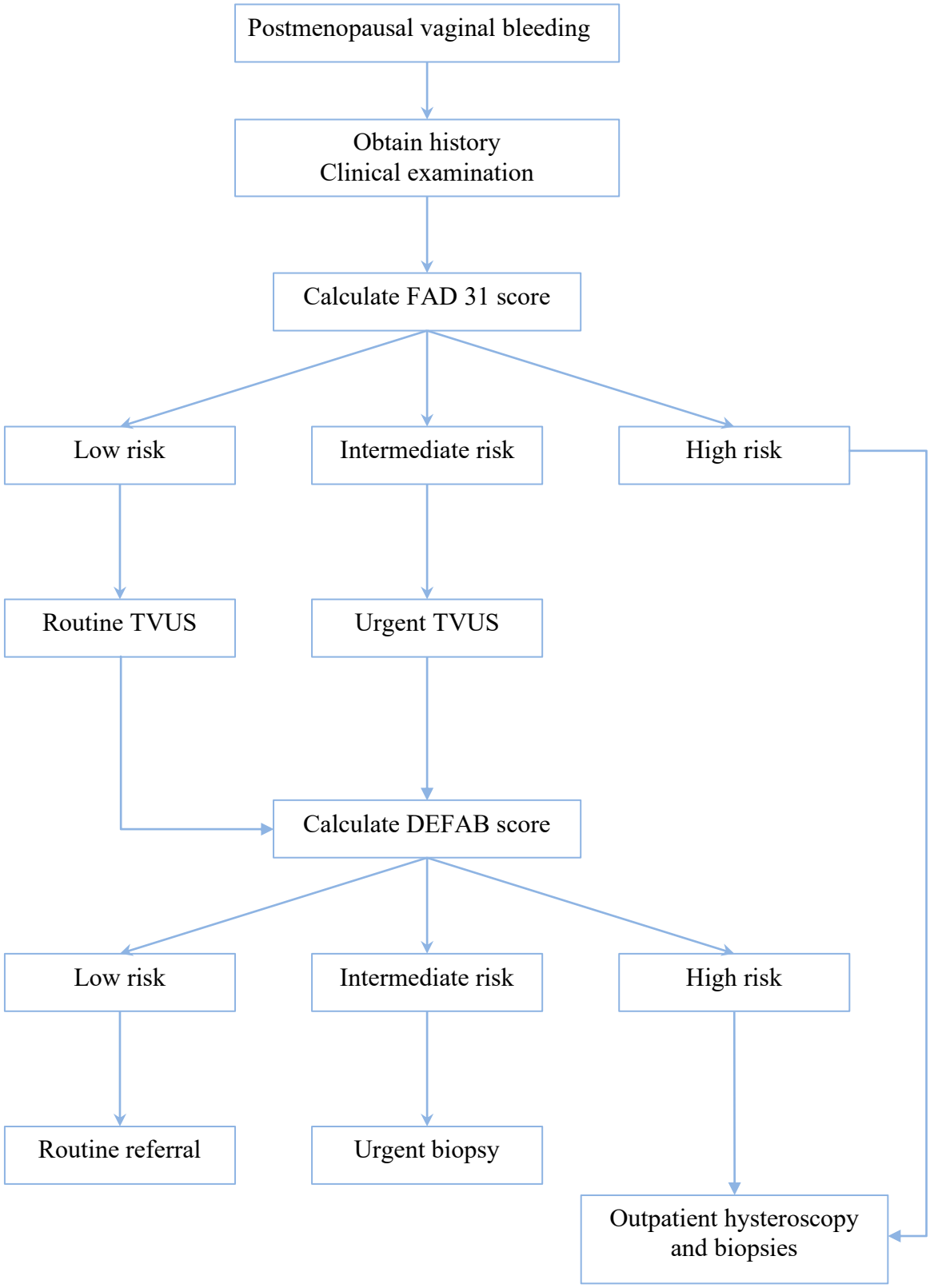
9.4 Potential clinical application of the predictive models

The discriminative ability of TVUS in women with PMB, as described by the area under the ROC curve, is reported to vary between 0.68-0.97 [280]. Despite the high sensitivity and specificity of TVUS as the initial test in women with PMB, in populations with low prevalence of endometrial cancer the positive predictive value of TVUS will be low.

The predictive models described in this thesis were developed with the aim of improving the risk stratification for women undergoing investigation for PMB. I envisage a two-step process for the management of these patients, as shown in flowchart 3. Patients presenting to primary care with PMB will undergo an initial assessment by their general practitioner. This will include obtaining a clinical history and performing physical examination. Based on data from the patients' clinical characteristics only, the FAD31 score will be calculated. The risk

of endometrial cancer will be estimated using a web-based medical calculator and patients will be classified as having low, intermediate or high risk of endometrial cancer. Patients at low risk of endometrial cancer can undergo a non-urgent TVUS, while patients at intermediate risk can be referred for an urgent TVUS. Following a TVUS and measurement of endometrial thickness, the DEFAB score is calculated and stratification of patients into low, intermediate and high-risk groups is performed for a second time. Patients at intermediate risk will be referred to undergo urgent biopsy in the outpatient setting while patients at low risk can wait longer. Patients at high risk of endometrial cancer at any stage of the assessment can be referred directly for hysteroscopy and biopsy. Such an approach can allow for individualised management, prioritisation of diagnostic tests and, consequently, more efficient use of resources.

Currently there are no agreed risk thresholds for classifying patients into different risk groups to guide clinical management [248]. A risk threshold should reflect the balance between the benefits of correct decisions against the costs of incorrect decisions [281]. However, optimal risk thresholds cannot be determined without estimating the performance of predictive models in external validation studies. Prevalence of the disease in the population, and sensitivity and specificity of the predictive model at each threshold may affect the choice of the optimal threshold [281]. In order to determine optimal risk thresholds, a health economic analysis should be performed following external validation studies [281].



Flowchart 3. Prioritisation of the referrals and diagnostic tests for women with PMB

Impact

As the models have not yet been externally validated, they cannot gain clinical impact in their own right. However, one Manchester-based study is currently attempting to externally validate the models [personal communication], and the research has provided an important foundation for the work of others. There is growing interest in the use of patterns of clinical symptoms - such as frequency of vaginal bleeding - as a predictor, and they have been incorporated in recently developed models [228, 233].

Citations (Search updated on May 25, 2021)

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Journal impact factor: 5.416

Number of citations: 51

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Number of citations: 32

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Number of citations: 15

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CiteScore: 0.550

Number of citations: 32

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CiteScore: 0.550

Number of citations: 16

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