A Simple Cervicovaginal Epigenetic Test for Screening and Rapid Triage of Women With Suspected Endometrial Cancer: Validation in Several Cohort and Case/Control Sets

Chiara Herzog, PhD1,2; Fátima Marín, PhD3,4; Allison Jones, BSc; Iona Evans, PhD5; Daniel Reisel, PhD5; Elisa Redl, MSc1,2; Lena Schreiberhuber, MSc1,2; Sonia Paytubi, PhD5; Beatriz Pelegrin, MSc1; Álvaro Carmona, PhD5; Paula Peremiquel-Trillas, MD6; Jon Frías-Gomez, MSc5; Marta Pineda, PhD5; Joan Brunet, MD, PhD3,4,7; Jordi Ponce, PhD5; Xavier Matias-Guiu, PhD4,9; Silvia de Sanjosé, PhD10; Laia Alemany, PhD6,11; Adeola Olatan, MD12; Michael Wong, PhD12; Davor Jurkovic, PhD12; Emma J. Crosbie, MD13,14; Adam N. Rosenthal, PhD5; Line Bjørge, PhD15,16; Michal Zikan, PhD17; Lukas Dostalek, MD, PhD18; David Cibula, PhD18; Karin Sundström, PhD18; Joakim Dillner, PhD19; Laura Costas PhD6,11; and Martin Widschwendter, MD1,5,2,20

abstract

PURPOSE Endometrial cancer (EC) incidence has been rising over the past 10 years. Delays in diagnosis reduce survival and necessitate more aggressive treatment. We aimed to develop and validate a simple, noninvasive, and reliable triage test for EC to reduce the number of invasive diagnostic procedures and improve patient survival.

METHODS We developed a test to screen and triage women with suspected EC using 726 cervical smear samples from women with and without EC, and validated the test in 562 cervicovaginal samples using three different collection methods (cervical smear: n = 248; vaginal swab: n = 63; and self-collection: n = 251) and four different settings (case/control: n = 388; cohort of women presenting with postmenopausal bleeding: n = 63; a cohort of high-risk women with Lynch syndrome: n = 25; and a nested case/control setting from a screening cohort and samples taken up to 3 years before EC diagnosis: n = 86).

RESULTS We describe the Women’s cancer risk IDENTification – quantitative polymerase chain reaction test for Endometrial Cancer (WID-qEC), a three-marker test that evaluates DNA methylation in gene regions of GYPC and ZSCAN12. In cervical, self-collected, and vaginal swab samples derived from symptomatic patients, it detected EC with sensitivities of 97.2% (95% CI, 90.2 to 99.7), 90.1% (83.6 to 94.6), and 100% (63.1 to 100), respectively, and specificities of 75.8% (63.6 to 85.5), 86.7% (79.3 to 92.2), and 89.1% (77.8 to 95.9), respectively. The WID-qEC identified 90.9% (95% CI, 70.8 to 98.9) of EC cases in samples predating diagnosis up to 1 year. Test performance was similar across menopausal status, age, stage, grade, ethnicity, and histology.

CONCLUSION The WID-qEC is a noninvasive reliable test for triage of women with symptoms suggestive of ECs. Because of the potential for self-collection, it could improve early diagnosis and reduce the reliance for in-person visits.

J Clin Oncol 40:3828-3838. © 2022 by American Society of Clinical Oncology

INTRODUCTION

Endometrial cancer (EC) is among the tumor types with the sharpest rising incidence over the past 10 years.1,2 Abnormal bleeding, defined as any postmenopausal, intermenstrual, or persistent heavy menstrual bleeding, is the lead symptom. EC patient survival is strongly dependent on stage at diagnosis,3 with delays in diagnosis and treatment resulting in significant adverse impacts on survival.4 The current route of diagnosis for suspected EC is transvaginal ultrasound (TVUS) followed by hysteroscopy and endometrial biopsy.2 During the COVID-19 lockdown, referrals via the 2-week-wait urgent pathway for suspected cancer in England, United Kingdom, decreased by up to 84%. A 3-month delay in EC diagnosis in England alone has been suggested to cause a loss of 6,305 life-years.6 A rapid triage modality that could rule out malignancies without the need for initial specialist referral could improve patient care and reduce time to diagnosis.

Current triage investigations available for suspected EC suffer from several limitations. Assessment of endometrial thickness using TVUS, the most frequently used initial investigation, is only feasible in postmenopausal women, and a cutoff of ≥ 5 mm has a sensitivity of 96.2% and specificity of 51.5%.7 In Black women, the performance is poor and offers a sensitivity...
of only 43.7%. A positive triage result needs to be followed up by histologic diagnosis, such as via hysteroscopic assessment and endometrial sampling. The low specificity of TVUS as an initial triage test, therefore, results in potentially high numbers of unnecessary invasive follow-up procedures. Alternatives to ultrasound investigation using molecular testing have been developed. A blood-based test using cell-free DNA sequencing has reported sensitivities of 28% and 16.7% for detecting overall and stage I uterine cancers, respectively. As summarized in our recent review, we and others have previously assessed DNA-based markers in cervicovaginal samples for EC detection. DNA methylation changes in cancer appear across an entire region rather than single sites, for example, all cytosine nucleotides followed by guanine nucleotides (CpGs) in a given CpG island may be hypermethylated. This results in a substantially increased signal-to-noise ratio compared with single point mutation analysis. Sensitivities using molecular methods in cervical samples reached ≈80%, but previous studies were affected by various issues, including low sample numbers (most studies included < 40 EC cases), lack of independent validation, low sensitivities, and no standardized test format that could be applied in samples obtained via different collection strategies.

A simple test to triage women with improved, or at least equivalent, accuracy to current standards (eg, TVUS) without the need for specialist referral is urgently needed. Ideally, such a test should also be applicable across different sample types, including self-collected samples. Here, we developed and applied the Women’s cancer risk IDentification – quantitative polymerase chain reaction test for Endometrial Cancer (WID-qEC) in several different settings. The test is based on quantitative, methylation-specific polymerase chain reaction (PCR) targeting one region in the gene ZSCAN12 and two regions in GYPC. Our data indicate that the WID-qEC may be amenable for use in self-collected samples and could improve triage and earlier diagnosis by reducing the need for in-person consultations and invasive testing caused by low specificity of TVUS. Future prospective studies comparing TVUS and the WID-qEC side-by-side will guide clinical application of this methylation-based test.

METHODS

Study Population

Development sets. We identified the most informative regions in an epigenome-wide screen in cervicovaginal specimens from 716 women and developed the PCR-based WID-qEC test in 40 individuals (FORECEE Pilot), 30 of whom were present in the epigenome-wide screen (total N = 726).

Validation sets. We validated the test in an independent group of 562 volunteers (all > 18 years) in three diagnostic and two predictive settings (Fig 1 and Table 1): (1) the FORECEE Validation set, consisting of cervicovaginal liquid-based cytology samples from asymptomatic women attending the hospital diagnosed as ECs (n = 71), benign gynecologic patients (n = 29), or healthy volunteers (n = 37), matched to cases by age; (2) the Barcelona Validation set, consisting of cervicovaginal self-samples from consecutive incident EC cases (n = 131), hospital controls with benign conditions (n = 102), and women attending hospital for nongynecologic diseases (n = 18), frequency-matched to cases by age; (3) the postmenopausal bleeding (PMB) Cohort, consisting of vaginal swabs from consecutive women presenting with postmenopausal bleeding at University College London Hospital (N = 63); (4) the Lynch Cohort, consisting of cervicovaginal liquid-based cytology samples collected from consecutive women presenting to University College London Hospital because of
Selected thresholds were identified using two predefined cutoffs: a high-risk, symptomatic threshold (66% sensitivity, 96% specificity) and a lower-risk threshold (25% sensitivity, 90% specificity). The test was then validated in five independent validation settings (continued on following page).
Lynch syndrome (N = 25); and (5) the Karolinska Cohort, a nested case/control setting using cervicovaginal liquid-based cytology samples collected between 2011 and 2015 from a Swedish cervical sample cohort—based biopsy bank from women diagnosed with EC up to 3 years after sample collection (n = 32) or women who did not develop EC by the end of the study period (2015) on the basis of the Swedish cancer registry (n = 54).

Detailed descriptions, including inclusion criteria and sampling methodology and STARD-2015 diagrams, are provided in the Data Supplement (online only).

Reference Test
For all EC cases, histology data following biopsy or hysterectomy were available to confirm diagnosis and deemed as the reference standard. For controls, histology was not always available. In addition to STARD diagrams, the Data Supplement details where confirmation of diagnosis using reference standard was available. Briefly, most controls in the FORECEE study did not undergo biopsy/histology, except for controls with benign gynecologic conditions. In the Barcelona Validation set, all controls with benign gynecologic conditions underwent biopsies while hospital controls (attending for other conditions) did not. In the PMB Cohort, only individuals with abnormal ultrasound underwent biopsies and histologic confirmation, while the rest without ultrasound abnormalities did not. All individuals in the Lynch Cohort, except for one participant who refused, underwent a biopsy and histologic assessment. In the Karolinska Cohort, only EC cases underwent biopsies.

WID-qEC DNA Methylation Assay
WID-qEC test regions were discovered in an epigenome-wide approach (approximately 850,000 methylation sites). Cervicovaginal samples from 572 controls and 144 women with EC (FORECEE study) were subjected to the Illumina MethylationEPIC array following a previously established pipeline.24 The epigenome-wide study and details of the methylation-specific PCR-based MethyLight assay11 are described in the Data Supplement.

DNA Mutation Analysis
Details of DNA mutation analysis are described in the Data Supplement. Briefly, DNA mutation analysis was performed for five genes (PTEN, TP53, PIK3CA, ARID1A, and CTNNB1) that led to the highest sensitivity of identifying cancers (ie, 92.9%) in The Cancer Genome Atlas data set, as previously described.25

Statistical Methods
Test performance was evaluated using sensitivity, specificity, and estimates of negative and positive predictive values (NPV/PPV). Details for statistical methods are presented in the Data Supplement. Original data are available in the Data Supplement, and all analysis code is available on github and is archived on Zenodo.26

RESULTS
WID-qEC Test Development
The workflow for WID-qEC test development and assessment is shown in Figure 1. A detailed overview of the test development and threshold selection is provided in the Data Supplement. For validation of the WID-qEC in five independent sets, two fixed thresholds were defined during test development: a high-sensitivity threshold (threshold 1) was applied in symptomatic and/or high-risk settings (FORECEE Validation, Barcelona Validation, PMB Cohort, and Lynch Cohort), whereas a high-specificity threshold (threshold 2) was applied in lower-risk settings (Karolinska Cohort) where it is important to limit false-positive results. We evaluated test performance in several sample sets representing clinically relevant potential applications and report the sensitivity, specificity, and estimated PPV and NPV in each setting. Analytical covariates include age, menopausal status (pre and post), ethnicity (White v non-White), and cancer characteristics (stage, histology, grade, and mutation mismatch repair [MMR] status).

WID-qEC in Cervical Smear Samples
Validation of the WID-qEC in 137 cervical smear samples (FORECEE Validation) led to a 97% sensitivity at 76% specificity and—assuming a prevalence of 9% in symptomatic women—PPV and NPV of 28% and 100%, respectively (Table 2). The results were similar, with overlapping confidence intervals, across ages, stages, grades, histologies, and menopausal status (Table 3, Data Supplement). Although sample numbers in non-White women were limited, WID-qEC performance did not seem to vary between Whites and non-Whites (Data Supplement).
### TABLE 1. Overview of Validation Data Sets

| Characteristic                  | Diagnostic | Predictive |
|--------------------------------|------------|------------|
|                                | FORECEE Validation | Barcelona Validation | PMB Cohort | Lynch Cohort | Karolinska Cohort |
|                                | Control (n = 66) | Control (n = 120) | Control (n = 55) | Control (n = 22) | Control (n = 54) |
|                                | EC (n = 71)     | EC (n = 131)   | EC (n = 8)      | EC (n = 3)       | EC (n = 32)      |
| Age, years, median (range)     | 65 (30-86)     | 68 (32-88)    | 66 (42-91)      | 58 (47-90)       | 58 (47-92)       |
|                                | 68 (50-86)     | 68 (32-88)    | 66 (42-91)      | 58 (47-90)       | 58 (47-92)       |
| Menopausal status, No. (%)     |              |              |                |                |                |
| Pre                            | 1 (1.5)       | 18 (15.0)     | 6 (4.6)         |                |                |
| Peri                           | 3 (2.5)       | 8 (6.1)       |                |                |                |
| Post                           | 65 (98.0)     | 98 (82.0)     | 117 (89.0)      | 55 (100.0)      | 22 (100.0)      |
| Unknown                        | 60 (91.0)     | 65 (92.0)     | 19 (15.0)       | 1 (33.0)        |                |
| Substrate LBC-TP Evalyn brush |              |              |                |                |                |
| Setting                        | Case/control  | Case/control  | Cohort          | Cohort          | Cohort          |
| Time to event, median (range)  | 0 (0-254)     | 755 (14-1,724)| 198 (2-1,095)   |                |                |
| Control diagnosis (controls), No. (%) |              |              |                |                |                |
| Normal, no diagnosis           | 37 (56.0)     | 120 (100.0)   | 131 (100.0)     | 55 (100.0)      | 120 (100.0)     |
| Unknown                        | 2 (3.6)       | 2 (3.6)       | 54 (100.0)      |                |                |
| Clinical grade (cases), No. (%)|              |              |                |                |                |
| Grade I                        | 16 (23.0)     | 53 (40.0)     | 51 (93.0)       | 3 (100.0)       |                |
| Grade II                       | 21 (30.0)     | 15 (11.0)     |                |                |                |
| Grade III                      | 29 (41.0)     | 62 (47.0)     |                |                |                |
| Unknown                        | 5 (7.0)       | 1 (0.8)       | 8 (100.0)       |                | 32 (100.0)      |

(continued on following page)
| Characteristic                      | Diagnostic | Predictive |
|------------------------------------|------------|------------|
|                                    | FORECEE Validation | Barcelona Validation | PMB Cohort | Lynch Cohort | Karolinska Cohort |
|                                    | Control (n = 66) | Control (n = 120) | Control (n = 55) | Control (n = 22) | Control (n = 54) |
| Cancer stage (cases), No. (%)      |             |             |             |             |                |
| I                                  | 40 (56.0)   | 92 (70.0)   | 3 (100.0)   |               |                |
| II                                 | 5 (7.0)     | 4 (3.1)     |             |               |                |
| III/IV                             | 22 (31.0)   | 34 (26.0)   |             |               |                |
| Unknown                            | 4 (5.6)     | 1 (0.8)     | 8 (100.0)   | 32 (100.0)    |                |
| Histology (cases), No. (%)         |             |             |             |             |                |
| Endometrioid                       | 47 (66.0)   | 94 (72.0)   | 6 (75.0)    | 3 (100.0)     |                |
| Serous                             | 11 (15.0)   | 19 (15.0)   |             |               |                |
| Clear cell                         | 3 (4.2)     | 3 (2.3)     |             |               |                |
| Other*                             | 6 (8.5)     | 15 (11.0)   | 2 (25.0)    |               |                |
| Unknown                            | 4 (5.6)     |             |             | 32 (100.0)    |                |

NOTE. We analyzed data sets from various sample collection devices (liquid-based cervical cytology, vaginal swab, and Evalyn brush), sample collection modalities (health care professional v self), and different patient groups (case/control, or cohort). Benign gynecologic conditions consisted of noncancerous gynecologic conditions, including endometriosis, benign cysts, fibromas, leiomyomas, prolapse, polyps, and evaluation for abnormal bleeding. These data sets enabled us to evaluate both the diagnostic and predictive use of the WID-qEC. Abbreviations: CAH, complex atypical hyperplasia; EC, endometrial cancer; HCP, health care professional; LBC-TP, liquid-based cytology (ThinPrep); PMB, postmenopausal bleeding. Other histologies include carcinosarcoma, mucinous, small cell, and squamous cancers.
TABLE 2. Sensitivity and Specificity of the WID-qEC

| Characteristic | Diagnostic | Predictive |
|---------------|------------|------------|
|               | FORECEE Validation | Barcelona Validation | PMB Cohort | Lynch Cohort | Karolinska Cohort |
| ECs, No.      | 71         | 131        | 8          | 3           | 32            |
| Cancer-free controls, No. | 66         | 120        | 55         | 22          | 54            |
| Sensitivity, % (95% CI) | 97.2 (90.2 to 99.7) | 90.1 (83.6 to 94.6) | 100.0 (63.1 to 100.0) | 33.3 (0.8 to 90.6) | 68.8 (50.0 to 89.9) |
| Specificity, % (95% CI) | 75.8 (63.6 to 85.5) | 86.7 (79.3 to 92.2) | 89.1 (77.8 to 95.9) | 100.0 (84.6 to 100.0) | 98.1 (90.1 to 100.0) |
| PPV, % (95% CI)* | 28.4 (20.5 to 37.8) | 40.1 (29.7 to 51.4) | 45.4 (27.4 to 60.2) | 50.7 (13.5 to 79.7) | 3.2 (0.5 to 19.1) |
| NPV, % (95% CI)* | 99.6 (98.6 to 99.9) | 98.9 (98.1 to 99.3) | 98.3 (92.3 to 99.4) | 92.2 (86.0 to 95.8) | 100.0 (100.0 to 100.0) |

NOTE. We assessed the WID-qEC in different clinically relevant settings. One of two predefined thresholds was used for evaluation (Data Supplement): the high-sensitivity threshold (threshold 1) was applied in symptomatic or high-risk settings (FORECEE Validation, Barcelona Validation, PMB Cohort, and Lynch Cohort), whereas the high-specificity threshold (threshold 2) was used for screening in lower-risk settings (Karolinska Cohort) to avoid false positives.

Abbreviations: EC, endometrial cancer; NPV, negative predictive value; PMB, postmenopausal bleeding; PPV, positive predictive value; WID-qEC, Women’s cancer risk Identification – quantitative polymerase chain reaction test for Endometrial Cancer.

*Assumed population prevalences: consecutively included women (PMB, cohort): 10.8%; symptomatic data sets (FORECEE, Barcelona Validation Set): 9%; asymptomatic/screening set (Karolinska Cohort): < 0.1%. Lynch cohort: 12%.

WID-qEC in Self-Collected Samples

When in-person physical access to medical facilities is restricted, a triage test ruling out malignancy would benefit from the option of sample self-collection. Among 251 (self-) Evalyn brush-collected samples (Barcelona Validation), 90% of ECs were correctly identified at 87% specificity (Table 2). The results were similar across different ages, menopausal statuses, or cancer stages, histologies, or grades (Table 3, Data Supplement). One hundred twenty-one women provided a truly self-collected sample, whereas for 130 women, health care professionals assisted with the collection using an Evalyn brush. WID-qEC performance was similar at detecting ECs in the two groups (Data Supplement).

For a subset of EC cases in this set (n = 109), DNA MMR status was available. The WID-qEC performed similarly in detection of MMR-proficient (n = 84) and MMR-deficient (n = 25) ECs (Data Supplement).

Comparison of WID-qEC, DNAmut, and Ultrasound

Ultrasound assessment is the most common initial assessment for suspected ECs, and DNA mutations in cervicovaginal samples were recently shown to indicate ECs.15 In the Barcelona Validation set, ultrasound and DNA mutation data were available for subsets of women in addition to the reference standard (histology; Data Supplement), which enabled an initial comparison of the WID-qEC with different triage modalities.

The WID-qEC offered similar sensitivity but significantly increased specificity compared with qualitative ultrasound assessment (ie, evaluation by the physician performing the ultrasound of normal or abnormal endometrial thickness; Data Supplement). AUCs for the WID-qEC score and quantitative ultrasound data (ie, endometrial thickness in millimeter) were similar (Fig 2A), but the WID-qEC exhibited higher specificity at high sensitivity than ultrasound on ROC curves (Fig 2A, Data Supplement). Moreover, compared with DNA mutation analysis (cutoff ≥ 1 mutation), the WID-qEC offered similar specificity but significantly increased sensitivity (Data Supplement) and a significantly higher AUC (Fig 2A). The same results (ie, higher specificity of WID-qEC compared with qualitative ultrasound data, and higher sensitivity compared with DNA mutation analysis) were exhibited in the analysis of stage 1 ECs only (Data Supplement). Taken

TABLE 3. WID-qEC Detects Exhibit Similarly High Sensitivity and Specificity Across Premenopausal and Postmenopausal Women and Histologic Differentiation

| Characteristic | Menopausal Status | Histology |
|---------------|-----------------|----------|
|               | Premenopause    | Postmenopause | Endometrioid | Serous |
| ECs, No.      | 7               | 187       | 141         | 30      |
| Cancer-free controls, No. | 19          | 163       | 186         | 186     |
| Sensitivity, % (95% CI) | 100.0 (59.0 to 100.0) | 92.5 (87.8 to 95.8) | 92.9 (87.3 to 96.5) | 96.7 (82.8 to 99.9) |
| Specificity, % (95% CI) | 78.9 (54.4 to 93.9) | 82.8 (76.1 to 88.3) | 82.8 (76.6 to 87.9) | 82.8 (76.6 to 87.9) |

NOTE. Analysis in samples of the FORECEE Validation and Barcelona Validation sets, for which data on menopausal state and histology were available. The high-sensitivity threshold (threshold 1) was applied.

Abbreviation: EC, endometrial cancer.
together, our data indicate that the WID-qEC outperformed DNA mutation analysis and performed at least equally well as ultrasound in the Barcelona Validation set.

**WID-qEC in a Consecutive Cohort of Women With PMB**

To assess the test in a real-life setting, we recruited 63 women who presented consecutively because of post-menopausal bleeding at an outpatient clinic (PMB Cohort). Health care professionals in the clinic collected a vaginal swab sample from the posterior fornix. The WID-qEC correctly identified 100% of women who were subsequently diagnosed with EC at 89% specificity (n = 8, Table 2).

**WID-qEC in a Cohort of Women With Lynch Syndrome**

To assess whether the WID-qEC identifies not only women with cancer, but also women at risk of developing cancer because of genetic predisposition, we analyzed a cohort of 25 women with Lynch syndrome who consecutively attended a surveillance clinic (Lynch Cohort). The WID-qEC was negative in all women who did not show any signs of cancer at the time of sample collection. In the single patient with a positive WID-qEC test (Data Supplement), the concurrent biopsy detected an endometrioid stage 1, grade 1 EC. The sensitivity of the WID-qEC was lower in this group compared with other settings (33%, Table 2). Interestingly, two out of three cancer cases also exhibited negative histology at the time of cervical sample collection.

**WID-qEC to Predict Future Cancer Risk**

Finally, we wanted to assess whether the WID-qEC detects EC in advance of current diagnosis. In the Karolinska Cohort, 69% of ECs were identified up to 3 years before diagnosis or follow-up in the Karolinska Cohort (Data Supplement). The applied threshold is the high-specificity threshold (threshold 2). AUC, area under the curve; DNAmut, DNA mutation; EC, endometrial cancer; PMR, percentage methylated reference; US, ultrasound; WID-qEC, Women’s cancer risk Identification – quantitative polymerase chain reaction test for Endometrial Cancer.

**DISCUSSION**

At the outset of this study, we aimed to develop a noninvasive EC screening and triage test. Here, we describe the WID-qEC, a simple three-marker DNA methylation–based test. We evaluate the test in clinically relevant diagnostic and predictive settings using various sample collection devices (cervical smear, vaginal swab, and self-collected...
samples. Applying prespecified thresholds across settings and collection modalities, our data indicate that the WID-qEC performs at least equally well, if not better, than other strategies currently in use to screen and triage women with EC, importantly ultrasound investigation.

Previous proof-of-concept studies, albeit using limited sample numbers, demonstrated the feasibility of discriminating between EC and benign conditions using genomic or epigenomic testing on Pap brush samples, tampons, or vaginal self-samples.11-17,19-21 Wang et al22 used a sensitive targeted sequencing method evaluating 18 genes of interest in a large case-control study (382 cancer cases and 714 controls): 81% of EC cases were identified in Pap brush samples. However, the specificity of this approach remains uncertain as controls were substantially younger than patients with EC (average age 34 v 62 years, respectively)22 and aging is strongly associated with the accumulation of somatic mutations.27 We used matched controls and observed high performance across study designs and populations. WID-qEC detection of ECs did not seem to vary by histology, grade, stage, age, ethnicity, and menopausal status in the cohorts studied here. However, future large-scale prospective studies will be required to further strengthen these data, in particular for covariates for which sample numbers in the current study were small (eg, premenopausal status, non-White ethnicities). Our data indicate that the WID-qEC could be suitable for use with a variety of collection devices. This, in combination with the high sensitivity and specificity of the test particularly in symptomatic settings, could make the WID-qEC test especially valuable in conditions where access to specialists or even any health care professional might be restricted (eg, global pandemic, nonurban settings, or in case of lengthy referral times).

The inherent limitations of case-control studies also apply to our study. We strived to mitigate bias by inclusion of several study designs (including cohorts) to ensure the robustness of the findings. We also included different sample collection methods to enhance generalizability and evaluate the potential of self-collection. Thresholds fixed during test development in the small FORECEE Pilot set detected ECs in all other sample sets and support the generalizability of our findings. A further limitation relates to the calculation of PPVs and NPVs, which were derived from the assumed population prevalence, meaning that changes in the assumed population prevalence could alter the estimated PPV/NPV. We note that although the PPV was sensitive to changes in estimated prevalence, the NPV remained relatively stable (Data Supplement). Nonetheless, diagnostic accuracy including PPV/NPV of the WID-qEC should ultimately be evaluated in large-scale prospective clinical studies in each target population. A limitation in the predictive evaluation using the Karolinska Cohort was that diagnoses beyond the end of 2015 were not known; hence, some controls could potentially have been diagnosed with EC after the last follow-up. Finally, we were unable to analyze 40/291 (13.7%) self-collected samples in the Barcelona Validation set because of insufficient DNA. Low yield is a common problem with self-sampling methods, but could be addressed with clear sampling guidance and optimized extraction protocols.

Two predefined diagnostic thresholds (high sensitivity or high specificity) were applied depending on the clinical context of each sample set. The appropriate threshold for clinical implementation depends on the setting: in low-risk populations, false positives should be avoided as they lead to unnecessary invasive procedures. Thus, a high-specificity approach is suitable (threshold 2). Conversely, in high-risk and/or symptomatic populations, high sensitivity is desired (threshold 1). Women who might benefit most from the WID-qEC in the near future might be (1) women presenting with abnormal bleeding or other symptoms suggestive of ECs undergoing triage for malignancies, in particular those for whom currently available tests (eg, ultrasound) are less reliable.8 For example, although the number of non-White women in our settings was low, the performance of the WID-qEC to detect EC was similar in White and non-White women; and (2) women at high risk of developing EC. The WID-qEC exhibited a high NPV in all settings.10 Pelvic ultrasound costs in the United States range from $220 US dollars (USD) to $3,200 USD, with a national average cost of $575 USD.28 As a relatively low-cost PCR-based test (estimated costs below $200 USD) with the potential for self-collection of samples, thus reducing the need for specialist referral, the WID-qEC offers several benefits compared with current clinical practice.

Initial clinical implementation of the WID-qEC test in circumstances where TVUS is inconclusive, not available, or declined by the patient is warranted to triage and prioritize patients with the highest cancer risk for hysteroscopy and endometrial biopsy. Given the potential benefits of earlier diagnosis of ECs for survival and reduced health care costs, future studies could also evaluate the test’s potential for screening of asymptomatic women with increased risk, for instance women with obesity or Lynch syndrome, or women in the general population age > 50 years who are participating in routine cervical screening.

In conclusion, the WID-qEC could represent a patient-friendly test for the screening and triage of women with symptoms suggestive of EC or those at risk of EC. Because of its suitability for use in self-collected samples, the WID-qEC may be a suitable tool for managing women with abnormal bleeding, particularly when access to specialist care is restricted. Further research will determine the most appropriate preventive screening and early detection settings in which to deploy this test.
Epigenetic Test to Triage Women With Suspected Endometrial Cancer

AFFILIATIONS
1European Translational Oncology Prevention and Screening (EUTOPS) Institute, Universität Innsbruck, Innsbruck, Austria
2Institute for Biomedical Aging Research, Universität Innsbruck, Innsbruck, Austria
3Hereditary Cancer Group, Catalan Institute of Oncology, IDIBELL, ONCOBELL Program, L’Hospital de Llobregat, Barcelona, Spain
4Cancer Epidemiology Research Programme, Catalan Institute of Oncology, IDIBELL, L’Hospital de Llobregat, Barcelona, Spain
5Hereditary Cancer Group, Catalan Institute of Oncology, IDIBGI, Girona, Spain
6Department of Gynecology, Hospital Universitari de Bellvitge, IDIBELL, Hospital de Llobregat, Barcelona, Spain
7Department of Pathology, Hospital Universitari de Bellvitge, IDIBELL, Hospital de Llobregat, Barcelona, Spain
8Supports from the European Union’s Horizon 2020 European Research Council Program under Grant Agreement No. 742432 (BRA-ERC) and the European Union’s Horizon 2020 research and innovation program under Grant Agreement No. 634570 (FORECEE) as well as the Land Tirol and the charity The Eve Appeal (https://eveappeal.org.uk/). The Barcelona study was conducted with the contribution of the Carlos III Health Institute through projects PIE16/00049, PI17/01179, and PI19/01835, as well as through CIBERONC CB06/02/0073 and CIBERONC CB16/12/00401, and CB16/12/00234, CM19/00216, FIZ200/0031, PID2019-111254R-B100, and MVZ2000029 cofinanced by the European Regional Development Fund ERDF, a way to build Europe. Furthermore, the support of the Secretariat for Universities and Research of the Department of Business and Knowledge of the Generalitat de Catalunya with grants to support the activities of research groups 2017SGR01085, 2017SGR01718, 2017SGR00735, and 2017SGR1282 and with funding from the Health Department of the Generalitat de Catalunya (PERIS SLT006177/6). E.I.C. is supported by the NIHR Manchester BRC (IS-BRC-1215-20007). A.N.R. was supported by the NIHR Biomedical Research Centre at University College London Hospitals National Health Service Foundation Trust and University College London.

AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
Disclosures provided by the authors are available with this article at DOI https://doi.org/10.1200/JCO.22.00266.

DATA SHARING STATEMENT
Epigenome-wide array data are deposited in the European Genome-Phenome Archive under the accession numbers EGAS00001005033 and EGAS00001005059. Data used for this manuscript are available in the Data Supplement, and analysis source code on GitHub (https://github.com/chiaraherzog/WID-qEC-source-code).

AUTHOR CONTRIBUTIONS
Conception and design: Laia Alemany, Adam Rosenthal, Michal Zikan, David Cibuła, Joakim Dillner, Laura Costas, Martin Widschwendter
Financial support: Line Bjørge, Joakim Dillner, Martin Widschwendter
Administrative support: Line Bjørge, Lukas Dostalek, Martin Widschwendter
Provision of study materials or patients: Sonia Paytubi, Paula Peremiquel-Trillas, Jon Frias-Gomez, Joan Brunet, Jordi Ponce, Xavier Matias-Guiu, Laia Alemany, Michael Wong, Davor Jurkovic, Adam Rosenthal, Line Bjørge, Michal Zikan, Joakim Dillner, Laura Costas, Martin Widschwendter
Collection and assembly of data: Fátima Marin, Allison Jones, Iona Evans, Daniel Reisel, Elisa Redl, Lena Schreiberhuber, Sonia Paytubi, Álvaro Carmona, Jon Frias-Gomez, Marta Pineda, Joan Brunet, Jordi Ponce, Xavier Matias-Guiu, Silvia de Sanjosé, Adeola Olaian, Michael Wong, Davor Jurkovic, Emma J. Crosbie, Line Bjørge, Michal Zikan, Lukas Dostalek, Karin Sundström, Joakim Dillner, Laura Costas, Martin Widschwendter
Data analysis and interpretation: Chiara Herzog, Fátima Marin, Iona Evans, Beatriz Pelegrina, Paula Peremiquel-Trillas, Marta Pineda, Silvia de Sanjosé, Laia Alemany, Davor Jurkovic, Emma J. Crosbie, Michelle Zikan, David Cibula, Karin Sundström, Laura Costas, Martin Widschwendter
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

ACKNOWLEDGMENT
The authors thank the CERCA Program/Generalitat de Catalunya for institutional support. E.I.C. is an NIHR Advanced Fellow (NIHR300650). The authors thank Javier de Francisco, José Manuel Martínez, Ilaria Bianchi, Francesc Xavier Bosch, Yolanda Benavente, Malcolm Scott, Sara Sleigh, and Nora Pashayan for their helpful contributions, and August Vidal and Axel Rodriguez for their support in sample management.
REFERENCES

1. Rahib L, Smith BD, Aizenberg R, et al: Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 74:2913-2921, 2014
2. Lortet-Tieulent J, Ferlay J, Bray F, et al: International patterns and trends in endometrial cancer incidence, 1978-2013. J Natl Cancer Inst 110:354-361, 2018
3. Lu KH, Broadrus RR: Endometrial cancer. N Engl J Med 383:2053-2064, 2020
4. Strohl AE, Feinglass JM, Shahabi S, et al: Surgical wait time: A new health indicator in women with endometrial cancer. Gynecol Oncol 141:511-515, 2016
5. Sundar S, Balega J, Crosbie E, et al: BGC1 uterine cancer guidelines: Recommendations for practice. Eur J Obstet Gynecol Reprod Biol 213:71-97, 2017
6. Sud A, Tott B, Jones ME, et al: Effect of delays in the 2-week-wait cancer referral pathway during the COVID-19 pandemic on cancer survival in the UK: A modelling study. Lancet Oncol 21:1035-1044, 2020
7. Long B, Clarke MA, Morillo ADM, et al: Ultrasound detection of endometrial cancer in women with postmenopausal bleeding: Systematic review and meta-analysis. Gynecol Oncol 157:624-633, 2020
8. Doll KM, Romano SS, Marsh EE, et al: Estimated performance of transvaginal ultrasonography for evaluation of postmenopausal bleeding in a simulated cohort of black and white women in the US. Jama Oncol 7:1158-1165, 2021
9. Klein EA, Richards D, Cohn A, et al: Clinical validation of a targeted methylation-based multi-cancer early detection test using an independent validation set. Ann Oncol 32:1167-1177, 2021
10. Costas L, Fries-Gomez J, Guardiola M, et al: New perspectives on screening and early detection of endometrial cancer. Int J Cancer 145:3194-3206, 2019
11. Fiegl H, Gattringer C, Widschwendter A, et al: Methylated DNA collected by tampons--a new tool to detect endometrial cancer. Cancer Epidemiol Biomarkers Prev 13:882-888, 2004
12. Doufekas K, Hadwen R, Kandimalla R, et al: GALR1 methylation in vaginal swabs is highly accurate in identifying women with endometrial cancer. Int J of Gynecol Cancer 23:1050-1055, 2013
13. Jones A, Teschendorff AE, Li Q, et al: Role of DNA methylation and epigenetic silencing of HAND2 in endometrial cancer development. PloS Med 10:e1001551, 2013
14. Kim GE, Kweon SS, Lee JS, et al: Quantitative assessment of DNA methylation for the detection of cervical and endometrial adenocarcinomas in liquid-based cytology specimens. Anal Quant Cytopathol Histopathol 34:195-203, 2012
15. Kinde I, Bettegowda C, Wang Y, et al: Evaluation of DNA from the Papanicolaou test to detect ovarian and endometrial cancers. Sci Transl Med 5:167ra4, 2013
16. Strooper LMD, Zummeren Mvan, Steenbergen RD, et al: CADM1, MAL and miR124-2 methylation analysis in cervical scrapes to detect cervical and endometrial cancer. J Clin Pathol 67:1067-1071, 2014
17. Bakkmun-Gamez JN, Wentzensen N, Maurer MJ, et al: Detection of endometrial cancer via molecular analysis of DNA collected with vaginal tampons. Gynecol Oncol 137:14-22, 2015
18. Wentzensen N, Bakkmun-Gamez JN, Killian JK, et al: Discovery and validation of methylation markers for endometrial cancer. Int J Cancer 135:1860-1868, 2014
19. Huang RL, Su PH, Liao YP, et al: Integrated epigenomics analysis reveals a DNA methylation panel for endometrial cancer detection using cervical scrapings. Clin Cancer Res 23:263-272, 2017
20. Chang CC, Wang HC, Liao YP, et al: The feasibility of detecting endometrial and ovarian cancer using DNA methylation biomarkers in cervical scrapings. J Gynecol Oncol 29:e17, 2018
21. Reijnem C, van der Putten LJM, Bulten J, et al: Mutational analysis of cervical cytology improves diagnosis of endometrial cancer: A prospective multicentre cohort study. Int J Cancer 146:2628-2635, 2020
22. Wang Y, Li L, Douville C, et al: Evaluation of liquid from the Papanicolaou test and other liquid biopsies for the detection of endometrial and ovarian cancers. Sci Transl Med 10:eaap8793, 2018
23. Wittenberger T, Sleigh S, Reisel D, et al: DNA methylation markers for early detection of women's cancer: Promise and challenges. Epigenomics 6:311-327, 2014
24. Barrett JE, Herzog C, Jones A, et al: The WID-BC-index identifies women with primary poor prognostic breast cancer based on DNA methylation in cervical samples. Nat Commun 13:449, 2022
25. Costas L, Palomero L, Benavente Y, et al: Defining a mutational signature for endometrial cancer screening and early detection. Cancer Epidemiol 61:129-132, 2019
26. Herzog C, Widschwendter M: WID-qEC Source Code, v1. Zenodo 10.5281/zenodo.6952787, as developed on GitHub, 2022. https://zenodo.org/record/6952787#.YvPE8z3MI2x
27. Milholland B, Auton A, Suh Y, et al: Age-related somatic mutations in the cancer genome. Oncotarget 6:24627-24635, 2015
28. New Choice Health: Pelvic Ultrasound Cost and Procedure Comparison. https://www.newchoicehealth.com/procedures/pelvic-ultrasound
AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

A Simple Cervicovaginal Epigenetic Test for Screening and Rapid Triage of Women With Suspected Endometrial Cancer: Validation in Several Cohort and Case/Control Sets

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript.

For more information about ASCO’s conflict of interest policy, please refer to www.asco.org/rcwc or ascopubs.org/jco/authors/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Chiara Herzog
Stock and Other Ownership Interests: Sola Diagnostics GmbH
Patents, Royalties, Other Intellectual Property: Named inventor on a patent combining epigenetic signatures for detection and risk stratification for breast cancer (WID-qtBC, Women’s cancer risk identification—quantitative trait Breast Cancer)

Paula Peremiquel-Trillas
Honoraria: Werfen
Travel, Accommodations, Expenses: Werfen

Joan Brunet
Consulting or Advisory Role: MSD Oncology, AstraZeneca Spain
Travel, Accommodations, Expenses: GlaxoSmithKline

Jordi Ponce
Consulting or Advisory Role: Medtronic, Abex, KCI

Xavier Matias-Guiu
Consulting or Advisory Role: AstraZeneca, Lilly, Amgen, Janssen, GlaxoSmithKline
Travel, Accommodations, Expenses: GlaxoSmithKline, Roche

Laia Alemany
Other Relationship: Integrated DNA Technologies (Inst), Roche (Inst)
Uncompensated Relationships: Roche (Inst)

Adam Rosenthal
Consulting or Advisory Role: Abcodia
Research Funding: Abcodia (Inst)

David Cibula
Consulting or Advisory Role: AstraZeneca, Sotio, Roche, GlaxoSmithKline, Novocure, Akeso Biopharma, MSD, Mersana

Karin Sundström
Consulting or Advisory Role: Merck (Inst)
Research Funding: Merck (Inst)

Laura Costas
Honoraria: Roche Sequencing Solutions
Other Relationship: Integrated DNA Technologies (Inst), Roche (Inst)
Uncompensated Relationships: Roche (Inst)

Martin Widschwendter
Patents, Royalties, Other Intellectual Property: I am a shareholder of Sola Diagnostics GmbH, which holds an exclusive license to the intellectual property that protects the commercialization of the WID tests. I am an inventor on pending patents describing the various WID tests. I am a shareholder of BreOva, which aims to license IP for developing and applying cell-free DNA methylation tests. I am an inventor on two pending patents on cell-free DNA methylation analysis for breast and ovarian cancer

No other potential conflicts of interest were reported.