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Molecular diagnosis of endometrial cancer from uterine aspirates

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Rapid and reliable diagnosis of endometrial cancer (EC) in uterine aspirates is highly desirable. Current sensitivity and failure rate of histological diagnosis limit the success of this method and subsequent hysteroscopy is often necessary. Using quantitative reverse transcriptase-polymerase chain reaction on RNA from uterine aspirates samples, we measured the expression level of 20 previously identified genes involved in EC pathology, created five algorithms based on combinations of

Key words: endometrial cancer, molecular diagnostics, endometrial aspirates

Abbreviations: AUB: abnormal uterine bleeding; EC: endometrial cancer; eCRF: electronic case report form; NPV: negative predictive value; PPV: positive predictive value; qRT-PCR: quantitative reverse transcriptase polymerase chain reaction; TVS: transvaginal ultrasound

Additional Supporting Information may be found in the online version of this article.

Conflicts of interest: Cristina Perez-Sanchez, Marta Palicio were and Alvaro Perdones-Montero, Eric Lalanne, Elena Aibar and Tamara Maes are currently employees of Oryzon genomics S.A. Tamara Maes is shareholder of Oryzon genomics. Elisabet Rosell-Vives was the first employee of Oryzon and currently of Reig Jofre group, as are Carlos Nieto and Alicia Ortega

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five genes and evaluated their ability to diagnose EC. The algorithms were tested in a prospective, double-blind, multicenter study. We enlisted 514 patients who presented with abnormal uterine bleeding. EC was diagnosed in 60 of the 514 patients (12%). Molecular analysis was performed on the remnants of aspirates and results were compared to the final histological diagnoses obtained through biopsies acquired by aspiration or guided by hysteroscopy, or from the specimens resected by hysterectomy. Algorithm 5 was the best performing molecular diagnostic classifier in the case–control and validation study. The molecular test had a sensitivity of 81%, specificity of 96%, positive predictive value (PPV) of 75% and negative predictive value (NPV) of 97%. A combination of the molecular and histological diagnosis had a sensitivity of 91%, specificity of 97%, PPV of 79% and NPV of 99% and the cases that could be diagnosed on uterine aspirate rose from 76 to 93% when combined with the molecular test. Incorporation of the molecular diagnosis increases the reliability of a negative diagnosis, reduces the need for hysteroscopies and helps to identify additional cases.

What's new?

Many studies report biomarker discovery using omics approaches, but few survive the translation into clinically validated diagnostic assays. Using previously identified biomarkers, here the authors set to improve the early diagnosis of endometrial cancer (EC) based on minimally invasive samples: endometrial aspirates. Current sensitivity and failure rate of histological diagnosis limit the success of aspirate-based diagnosis and subsequent hysteroscopy is often necessary. The authors developed and clinically validated a novel molecular test, which increases the efficacy, sensitivity and negative predictive value of aspirate-based diagnosis and has the potential to reduce the average time and cost for EC diagnosis.

Endometrial cancer (EC) is the second most frequent gynecological cancer worldwide. Approximately 300,000 new cases are diagnosed every year, accounting for almost 8.2% of the worldwide incidence of cancer in women.[1] In developed countries, EC is the most frequent gynecological cancer, continuing to increase as a consequence of aging and growth in the population and the adoption of cancer-associated lifestyle choices.[2] EC is highly curable in its initial stages with an overall 5-year survival rate of 96%.[3] However, its delayed detection is an important contributor to increased disease stage and, hence, a higher rate of mortality.[4]

Focusing on early detection, the International Federation of Gynecology and Obstetrics (FIGO)[5] first aims to adequately inform women at risk[6–8] to report any unexpected abnormal uterine bleeding (AUB) or spotting to their physician. AUB is the most frequent indicator of EC, but many other benign disorders give rise to the same symptom.[9] When confronted with clinical suspicion of EC, the current diagnostic cornerstone is the histological analysis of an endometrial biopsy. Biopsies are preferably obtained by a minimally invasive office procedure using a flexible, disposable endometrial suction curette or pipelle that permits the aspiration of endometrial fluid from inside the uterine cavity. However, this methodology presents limitations. A failure rate of 8% has been reported in obtaining samples, while 13% of the samples obtained turn out to be histologically inadequate. In addition, failure rates rise in postmenopausal women.[10]

A biopsy guided by hysteroscopy may also be performed. The histological analysis of samples obtained by hysteroscopy is reported to be more sensitive than that of samples obtained by aspiration.[11–13] However, patients who undergo hysteroscopy are at an increased risk of complications, including uterine perforation, hemorrhage and possible harm to other organs, as well as stroke and intoxication provoked by the dilation procedure.[14] This procedure has also been associated with the peritoneal dissemination of EC cells, although no direct link between hysteroscopy and the prognosis of the disease has been found.[15]

To date, several studies have been conducted to profile EC at the molecular level[16–18] with the aim of improving the sensitivity of current diagnostic methods. However, none of the biomarkers or profiles identified has been extensively validated, nor have any been translated to clinical utility.

Here, we report the development and clinical validation of a novel molecular assay to detect EC, based on five differentially expressed biomarkers identified previously in a genome-wide gene expression study[19] on EC. We first developed reliable quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) assays to measure the expression levels of the genes and created five diagnostic algorithms for EC using a case–control population. Next, we validated the algorithms in a double-blind, multicenter study and describe here how the test can be used to potentiate EC diagnosis on endometrial aspirates.

Material and Methods

Study design and population

Case control study population. To develop the molecular diagnostic classifiers a case–control study was performed on uterine aspirate samples obtained from 71 consecutive patients, 23 of whom were diagnosed with EC in the Department of Gynecological Oncology at Vall d'Hebron University Hospital. A detailed description of the case–control study population is provided in Table 1.

Table 1. Baseline characteristics of the patients

Case–control study	Endometrial cancer ($n = 23$)	Other diagnosis ($n = 48$)
Age (years)		
Median	64	50
Minimum	32	22
Maximum	88	85
Uterine condition		
Premenopausal: secretory phase	–	5
Premenopausal: proliferative phase	–	4
Postmenopausal	16	19
Unclassified	7	20

	All patients ($n = 514$)		Patients with molecular diagnosis ($n = 372$)	
Validation study	Endometrial cancer ($n = 60$)	Other diagnosis ($n = 454$)	Endometrial cancer ($n = 47$)	Other diagnosis ($n = 325$)
Age (years)				
Median	66	52	66	52
Minimum	47	45	51	45
Maximum	86	90	86	83
Uterine condition				
Premenopausal: secretory phase	2	106	1	81
Premenopausal: proliferative phase	3	75	2	59
Postmenopausal	55	273	44	185
Unclassified	–	–	–	–
Endometrial cancer	60	–	47	–
Typology—*n* (%)				
Type I	47 (78)	–	38 (81)	–
Type II	10 (17)	–	7 (15)	–
Unclassified	3 (5)	–	2 (4)	–
Histologic grade—*n* (%)				
Grade 1	24 (40)	–	23 (49)	–
Grade 2	24 (40)	–	15 (32)	–
Grade 3	8 (13)	–	6 (13)	–
Unknown	1 (2)	–	1 (2)	–
Unclassified	3 (5)	–	2 (4)	–
FIGO stage—*n* (%)				
IA	43 (71)	–	35 (74)	–
IB	6 (10)	–	5 (11)	–
IIIB	3 (5)	–	1 (2)	–
IIB	5 (8)	–	4 (8)	–
Unclassified	3 (5)	–	2 (4)	–
Myometrial invasion—*n* (%)				
<50%	45 (75)	–	36 (77)	–
>50%	12 (20)	–	9 (19)	–
Unclassified	3 (5)	–	2 (4)	–

Table 1. Baseline characteristics of the patients (Continued)

	All patients ($n = 514$)		Patients with molecular diagnosis ($n = 372$)	
Validation study	Endometrial cancer ($n = 60$)	Other diagnosis ($n = 454$)	Endometrial cancer ($n = 47$)	Other diagnosis ($n = 325$)
Lymph-vascular permeation—*n* (%)				
Yes	44 (73)	–	34 (72)	–
No	13 (22)	–	11 (24)	–
Unclassified	3 (5)	–	2 (4)	–
Other clinical findings				
Endometrial polyps—*n* (%)				
Yes	9 (15)	112 (25)	7 (15)	76 (23)
No	51 (85)	342 (75)	40 (85)	249 (77)
Hyperplasia—*n* (%)				
Yes	7 (12)	6 (1)	6 (13)	5 (1)
No	53 (88)	448 (99)	41 (87)	320 (99)
Endometrial thickness (mm)				
Mean	16	8	14	8
Interquartile range	9–20	4–11	8–20	4–11

RNA isolation and development of the prototype qRT-PCR assay

Total RNA was extracted from pelleted aspirate samples with the RNeasy mini Kit or RNeasy MinElute Cleanup Kit (Qiagen, Valencia, CA). RNA concentration and quality were assessed by Nanodrop ND-1000 spectrophotometer and Agilent 2100 bioanalyser RNA (Agilent, Santa Clara, CA), respectively. Samples with less than 0.5 μg of total RNA extracted were deemed insufficient/invalid for qRT-PCR analysis. For quantitative RT-PCR (qRT-PCR), first-strand cDNA was synthesized from 0.5 to 1 μg of total RNA using the High Capacity RNA-to-cDNA Master Mix from Applied Biosystems (Life Technologies, Foster, CA) (Cat# 4390779). Forward and reverse primers for qRT-PCR were designed using the Primer3 program.[20] Hydrolysis probes were obtained from Roche (Hoffmann La-Roche, Nutley, NJ). Transcripts were amplified with reagent (LightCycler® 480 Probes Master, Roche) in a LightCycler® 480 II System (Roche). qRT-PCR analysis was done in 96-well plates in a 20 μl volume using the cDNA equivalent to 20 ng of total RNA per well. PCR reactions were run in triplicate, and mean values were used for analysis to ensure reproducible results. Variability among the plates was normalized using a control cDNA as a reference value for each plate. Gene expression was quantified in relation to the expression of an endogenous control.

Development of classification models

Classification models were generated to translate the levels of expression of the five assessed by qRT-PCR into a categorical EC cancer score. The sample set included aspirates from 71 patients, 23 of whom were diagnosed with EC diagnosis. Selected biomarker combinations were used to generate the diagnosis predictors. Different classification models such as support vector machine (SVM), decision tree forest, neuronal networks or linear regression were used to calculate the distinct diagnosis predictors and were validated using the ten-fold cross-validation or leave-one out methods. To avoid overfitting, we used a pruning method called minimum error to calculate the diagnosis predictors. The flowchart for the development and validation of the molecular classifiers is depicted in Figure 1.

Clinical validation study

To validate and select the best classifier to diagnose EC, a prospective, double-blind, multicenter study (GEAKIT-09) was performed. Fourteen different hospitals across Spain participated in the study, ten of which were members of REDITOG, a hospital translational molecular biology network that was created to provide support for basic research by participating in studies at different clinical stages. In our study, 514 patients were recruited between December 2009 and September 2010. Inclusion criteria were a minimum age of 45 years and AUB. Women who had been treated previously for gynecological pelvic cancer were excluded. Patients known to be positive for the human immunodeficiency virus and/or the hepatitis virus were excluded for safety reasons. The patients enrolled in both studies provided a written informed consent, and the institutional review boards of each participating hospital approved the protocol.

Clinical and personal data from the patients were securely collected by clinicians and recorded in an electronic case report form (eCRF). Similarly, molecular data from the

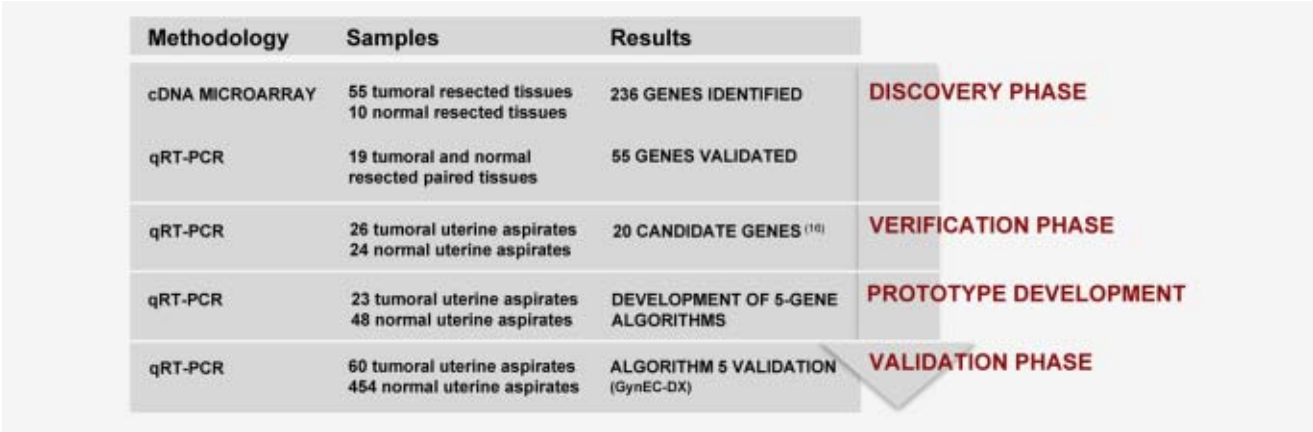

Figure 1. Flow chart for the development of the molecular test to identify EC on uterine aspirate samples. Briefly, a description of each method used for the discovery or validation of the selected candidates and a description of the samples is specified for every phase of the project. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

analysis of samples were recorded in this eCRF by the central laboratory at Oryzon Genomics. During the course of these studies, neither the clinicians nor the central lab had access to the data from the other part. The eCRF was closed in February 2011 and the data were unlocked for analysis. A detailed description of the study population is provided in Table 1.

Sample collection and molecular analysis

Clinicians collected uterine aspirate samples for histological analysis using an endometrial suction curette from Gynetics Medical Products (Cat# 4164). The remnants of these samples were deposited into microtubes containing RNA-preserving solution, pelleted, frozen on dry ice and sent to a reference laboratory for molecular analysis. RNA extraction and qRT-PCR were performed as in the case–control study.

Diagnostic procedure and adjudicated final diagnosis

The current guidelines from FIGO[5] were followed to determine the final diagnosis for each patient and to classify the type and grade of the primary tumors found in women with a diagnosis of cancer. All enrolled patients underwent pelvic examination and transvaginal ultrasonography. The diagnostic algorithm for EC is heterogeneous; in most hospitals endometrial sampling by aspiration was performed before hysteroscopy but some hospitals preferred hysteroscopy as the first-line sampling method. The validation study allowed us to quantitatively assess the flow of patients through the current diagnostic algorithm. In the validation population, 456 patients underwent endometrial sampling by aspiration for histology. Of these patients, 168 were subsequently submitted to hysteroscopy (132 with a negative, ten positive and 26 inconclusive aspirate histology results). Forty-one patients were analyzed directly by hysteroscopy, and 77women underwent surgery.

To achieve the final clinical diagnosis, a histological analysis was performed on the biopsies obtained by aspiration, on biopsies guided by hysteroscopy and/or on the specimens resected by hysterectomy. When several histological analyses were performed on the same patient, the diagnosis attributed to the more invasive sample type analyzed prevailed. Although positive diagnoses are generally highly reliable and contrasted by higher level analyses, it must be understood at all times that negative results are often not contrasted.

The molecular test was grafted on the protocol implemented at each site and performed either on the remnants of the aspirate samples obtained for histological analysis or on an aspirate sample obtained during the visit for hysteroscopy. The adjudicated final diagnosis was taken as the reference standard.

Statistical analyses

The primary end point of the GEAKIT-09 study was to determine the diagnostic accuracy of five different classifiers for EC using uterine aspirate fluid samples for the analysis, taking the final diagnosis as a reference. Additional ends points included the comparison of the sensitivity and specificity of the molecular diagnosis with the histological analysis of endometrial aspirate fluid.

For each endometrial sample analyzed during the clinical validation, each molecular classifier provided a score that was calculated from the normalized expression levels of five EC biomarkers. After the cutoff established in the case–control study was applied, a categorical diagnosis ("cancer" *vs.* "not cancer") was made and the results recorded in the eCRF.

Categorical diagnoses provided by each of the five evaluated classifiers were compared to the adjudicated final diagnosis (reference standard) using two-by-two tables and the respective negative (NPV) and positive predictive values (PPV), sensitivities and the specificities were calculated. An analysis of receiver-operating-characteristic (ROC) curve was also calculated for each classifier and for the individual biomarkers.

Table 2. Diagnostic performance of the molecular classifiers

	Algorithm 1	Algorithm 2	Algorithm 3	Algorithm 4	Algorithm 5
	Percent (95% CI)				
Case–control study (71 patients)					
Sensitivity	100 (82–100)	100 (82–100)	100 (81–100)	100 (81–100)	100 (81–100)
Specificity	92(79–97)	62 (47–76)	98 (88–100)	91 (78–97)	98 (87–100)
Positive predictive value	85(65–95)	56 (40–71)	95 (75–100)	84 (63–95)	95 (75–100)
Negative predictive value	100 (90–100)	100 (86–100)	100 (91–100)	100 (90–100)	100 (90–100)
Validation study (372 patients)					
Sensitivity	79 (64–89)	83 (69–92)	62 (46–75)	64 (48–77)	81 (66–90)
Specificity	93 (90–96)	92 (88–94)	95 (92–97)	93 (89–95)	96 (93–98)
Positive predictive value	63 (49–75)	59 (46–71)	63 (47–76)	57 (42–70)	75 (60–85)
Negative predictive value	97 (94–98)	97 (95–99)	94 (91–97)	95 (91–97)	97 (95–99)

Results

Development of the assay prototype and classification models in a case–control study

To develop a cost-viable assay prototype, it was necessary to translate the biomarkers identified previously in higher content assays[19] to a format designed to analyze a small profile consisting of the best performing individual markers. The six best performing markers identified previously in the genome-wide study (criteria for individual marker selection p-value < 0.0001; SVM AUROC > 0.88) were successfully translated to the individual assay format with exception of the RNF183 assay that was discarded because of inefficient PCR performance in a cell line reference sample. The qRT-PCR assays were then performed on endometrial aspirate samples obtained from the case–control population and the gene expression data from the five individual markers were combined to develop five robust multivariate classification models for EC diagnosis. The ROC curves for the five algorithms in the case–control study are represented in Supporting Information Figure S1A.

Clinical validation study

A total of 514 women met all of the inclusion criteria and were eligible for enrollment in the study. Sixty women (11.7%) were diagnosed with EC. The distribution of cancer subtypes found (83.3% were Type I EC and 16.7% Type II EC) was in keeping with the expected prevalence of both subtypes.[20] The rest of the population suffered AUB because of medical conditions other than EC. Complete descriptions of the characteristics of the study population are listed in Table 1.

The pelleted remnants of the 514 uterine aspirate samples yielded sufficient RNA for molecular diagnosis in 372 cases (72.4%), of which 47 were diagnosed with EC (12.6%) and 325 were diagnosed as negative using the standard histological diagnosis.

Performance of molecular classifiers in the clinical validation study

We examined the performance of the five classifiers in the 372 uterine aspirates that yielded sufficient RNA for molecular analysis and calculated the ROC curves for each of them (Supporting Information Fig. S1B). A summary of the statistical parameters for sensitivity, specificity, PPV and NPV for each classifier test is shown in Table 2. The likelihood ratios, which depend on the sensitivity and specificity, but not the prevalence of the disease, were also calculated.

When compared to the reference standard in our study, defined as the final diagnosis based on histology derived from the highest level analysis from endometrial aspirate, hysteroscopy-guided biopsy or analysis of the surgical piece; all of the algorithms presented a high NPV. Algorithm 2 presented an NPV of 97% [95% confidence interval (CI): 95–99%), the highest of all five algorithms. It was also the algorithm with the highest sensitivity at 83% (95% CI: 69–92%) and the smallest negative likelihood ratio at 0.19 (95% CI: 0.10–0.35). Algorithm 2 produced the fewest false negatives and was the one that most reduced the odds ratio of EC when the test result was negative. However, the algorithm with the highest specificity was algorithm 5 at 96% (95% CI: 93–98%). The NPV and the negative likelihood ratio of this algorithm were practically identical to the NPV of algorithm 2, but the positive likelihood ratio was considerably higher for algorithm 5 when compared to algorithm 2 at 20.2 (95% CI: 11.7–35.1) *versus* 10.0 (95% CI: 6.8–14.6). This implied a significant increase of the EC odds ratio when the diagnostic result of algorithm 5 was positive. Judging from the likelihood ratios and confirming the results obtained on the case–control population, the best performing molecular diagnostic algorithm in the GEAKIT-09 study was algorithm 5 (Table 2). This algorithm was used for all the comparisons and combinations described below.

Comparison and combination of molecular and histological diagnoses

Of the 514 patients that underwent the standard protocol of diagnosis, 456 patients (89%) were subjected to biopsy by aspiration and 209 (41%) to biopsy guided by hysteroscopy. Histological examination of the uterine aspirates gave

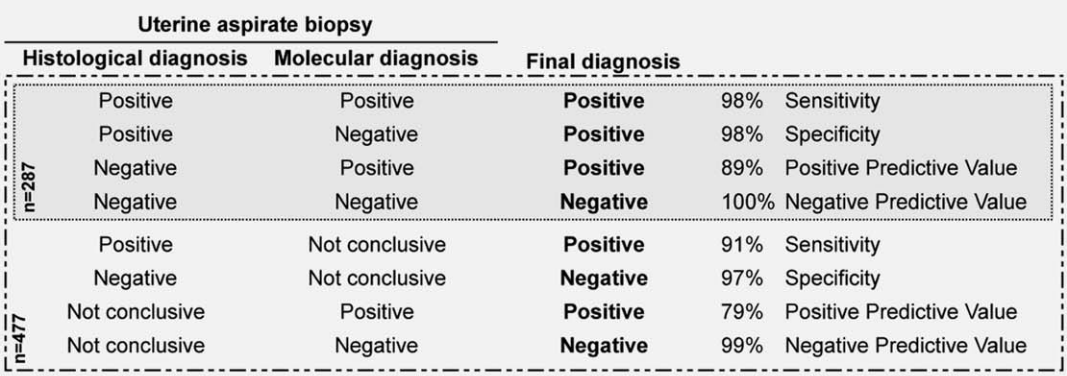

Figure 2. A combined analysis between the histological and molecular assays was done on uterine aspirates. Applying the criteria "either positive," a positive final result was assigned as soon as one of the screening tests was positive. Two different subpopulations were considered. The first one ($n = 287$) corresponded to the samples which obtained a conclusive results from both assays. The second one ($n = 477$) considered those samples which obtained a conclusive result from either one or both assays. A "positive" means that the sample was diagnosed as EC and "negative" that was diagnosed no EC.

informative results for 392 of the 456 cases (86%), and an overall sensitivity and specificity of 85 and 99%, respectively. Histological examination of the biopsies guided by hysteroscopy gave informative results in 200 of the 209 cases (96%) and showed excellent sensitivity and specificity, 91 and 100%, respectively. Molecular diagnosis reported a good sensitivity and specificity, 81 and 96% for samples for which sufficient RNA was available ($n = 372$, 72%).

All cases classified as nonatypical endometrial hyperplasia ($n = 27$) or proliferative endometrium ($n = 15$) by histology in our study were deemed negative by molecular diagnostics. Five of 11 cases of atypical complex endometrial hyperplasia were classified as positive.

As most cases with a negative aspirate histology are not further contrasted by next-level analysis (which skews the diagnostic performance in favor of the histological method), the performance of the molecular test was also evaluated on the subpopulation analyzed by hysteroscopy ($n = 152$; 16 EC cases). Sensitivity rose to 94% (all EC cases detected by hysteroscopy were also detected by molecular analysis), NPV was 99% and the PPV was 94%. *Vice versa*, the histological diagnosis on aspirates in the subpopulation that was analyzed by hysteroscopy ($n = 142$; 18 EC cases) had a sensitivity of only 55% and the NPV was 94% (this subpopulation logically concentrated cases with false-negative results for EC in the aspirate histology), although the PPV was 100%.

The percentage of cases for which informative results were obtained from uterine aspirates was similar in the molecular diagnostic and the histological examination (372 *versus* 392 cases) but the two populations only partially overlapped (287 cases). We evaluated a combination of the molecular and histological analysis on biopsies obtained by aspiration (histology U molecular test; either test positive), assigning a positive final diagnosis for EC as soon as one assay on the sample was positive. Following this new model (Fig. 2), the informative results were obtained in 477 of the 514 cases (93%), and they presented a sensitivity of 91%, a specificity of 97%, a NPV of 99% and a PPV of 79%. When results from both histological and molecular tests were available (histology ∩ molecular test; either test positive), the analyses on aspirates exhibited superior power to diagnose EC with a sensitivity, specificity and NPV of 98, 98 and 100%, respectively; yet it has only 287 patients (63%) could be diagnosed by both methods. The PPV of this combination was 89%, exclusively because of false positives from the molecular test.

All comparisons were made to the final clinical diagnosis as described in the Material and Methods section and are presented in Table 3.

Discussion

A prospective, double-blind, multicenter study was designed to compare the performance of a novel molecular diagnostic assay for the detection of EC with the standard histology-based procedures. In the validation study, 514 women aged 45 years or older and presenting with AUB were enrolled. In this particular age stratum (premenopause and postmenopause), the vast majority of women with an AUB do not have EC and it is essential to rule out EC as soon as possible with a minimal discomfort to the patients.

The first step in the evaluation of patients presenting with AUB is the measurement of the endometrial thickness by transvaginal ultrasound (TVS). According to some authors TVS has a good sensitivity for EC diagnosis in postmenopausal women, but other reports are less supportive.[21–23] Even if TVS aids to identify patients at risk, further testing is warranted to diagnose EC.

Current methods to diagnose EC are based on histological examination of endometrium biopsies. It is well known that for the diagnosis of EC, histology performed on samples obtained by hysteroscopy outperforms histology on blind endometrial aspirates in efficacy, sensitivity and NPV. The data obtained from our study confirm this finding. Not

Table 3. Diagnostic performance of different tests

Sample type Test method	Aspirate fluid Ecography >4 mm	Histology	Algorithm 5	Histology and/or Algorithm 5	Histology and Algorithm 5	Biopsy guided by hysteroscopy Histology
Number of patients (*n*)	488	392	372	477	287	200
Variables percent	Percent (95% CI)					
Sensitivity	93 (82–98)	85 (72–93)	81 (68–90)	91 (80–97)	98 (86–100)	91 (69–98)
Specificity	26 (22–30)	99 (98–100)	96 (93–98)	97 (94–98)	98 (95–99)	100 (97–100)
Positive predictive value	14 (11–18)	98 (87–100)	75 (60–85)	79 (67–88)	89 (75–96)	100 (80–100)
Negative predictive value	97 (91–99)	98 (95–99)	97 (95–99)	99 (97–100)	100 (97–100)	99 (96–100)

surprisingly, we found that 38% of the patients with a negative aspirate biopsy result were subsequently subjected to hysteroscopy, highlighting a lack of confidence of the gynecologists in the adequacy of this method to exclude EC. Effectively, 6% of these aspirate biopsy-negative patients were diagnosed with EC using next-level analyses, which means the probability of having EC after receiving a negative aspirate histology result was reduced only twofold in comparison to that in the initial AUB-suffering population analyzed in our study.

Hysteroscopy is often viewed as the gold standard to identify EC; yet few studies actually provide data that allow the direct comparison of the diagnostic capacity of the biopsy guided by hysteroscopy with that of examination for EC in hysterectomy specimens.[13] In addition, it is becoming clear that even hysteroscopy and total curettage are not sufficient to discriminate atypical endometrial hyperplasia from EC.[24,25]

Thus, while histology on the endometrial aspirate represents a minimally invasive diagnostic method and is highly attractive for early diagnosis and while hysteroscopy is considered a more reliable although more invasive method, there remains no consensus on the most efficient diagnostic pathway.[26]

The diagnostic performance of the molecular test on the endometrial aspirate was evaluated using the final diagnosis derived from the highest level analysis from endometrial aspirate, hysteroscopy-guided biopsy or analysis of the surgical piece as a reference. Performance was similar to that of the histological analysis of uterine aspirates (sensitivity, specificity, PPV and NPV at 81, 96, 75 and 97%, respectively, and a positive likelihood ratio of 20.2). The sensitivity and NPV of the molecular test on the aspirate sample were higher (94 and 99%) in the subpopulation of patients submitted to hysteroscopy; all EC cases detected by hysteroscopy were detected by molecular analysis.

The combination of the histological and molecular analyses of uterine aspirates (histology U molecular test; either test positive) significantly increased the sensitivity (91%), specificity (97%) and NPV (99%) of EC diagnosis and also increased the total number of patients that could be diagnosed using endometrial aspiration from 392 to 477. The sensitivity and NPV of the combined molecular and histological analyses on the endometrial aspirate samples were higher than that of histology on endometrial aspirates alone, and equaled that of hysteroscopy.

The molecular test permitted the identification of six EC cases that were erroneously classified as negative or inconclusive by histology on uterine aspirate samples, one case missed by hysteroscopy and one case detected after surgery. On the other hand, the test produced 13 false-positive cases. Of these, seven were confirmed as negative by histological analysis of biopsies obtained by hysteroscopy but six were not contrasted further. Therefore, the inclusion of the molecular test could help to identify additional cancer patients but would only marginally increment the number of patients derived unnecessarily to hysteroscopy; while it could substantially reduce the number of patients requiring next-level analysis to exclude EC.

To summarize, our goal was to improve the early diagnosis of EC with the development of a test that could exclude the disease in a fast and reliable manner using aspirate biopsies, and that would have the potential to provide relief from physical and psychological distress to patients and to reduce the number of interventions required for reaching cancer diagnosis. Here, we have shown that we can meet this objective by the introduction of a novel molecular test, from hence on termed GynEC®-DX, that increases the efficacy, sensitivity and NPV of the diagnosis on endometrial aspirates and has the potential to reduce the average time and cost to diagnose EC in women with AUB.

Further studies will reveal if the test also has utility in asymptomatic postmenopausal women with increased endometrial thickness—if reasonable criteria for TVS can be defined to triage these women for further analysis[27,28]—or for women at increased risk for EC including patients with Lynch syndrome[29] or women submitted previously to tamoxifen treatment.[30]

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