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Research Article

Validation of New GIMA Biomarker Signature of Endometriosis-Interim Data

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ABSTRACT

Objective: Validate diagnostic accuracy of new unique biomarker, Gastrointestinal Myoelectrical Activity (GIMA), detected by Electroviscerography (EVG) with Ai-derived disease threshold score calculation to non-invasively diagnose endometriosis.

Design: Multicenter prospective blinded trial.

Setting: Women's healthcare center.

Population of Sample: 165 patients with and without endometriosis diagnosis.

Methods: Initial 50 patients meeting inclusion criteria in 165-patients multicenter prospective GIMA biomarker trial were selected for interim analysis. Study population included women 27 years-55 years old, 25 with diagnosis of endometriosis and 25 non-endometriosis controls. Clinical and GIMA data were collected between February 2007 and September 2017, at all harvesting time points and frequency bands using EVG. Ai-derived threshold score calculations used Area Under The Curve (AUC), age and standardized pain scores variables.

Main Outcome Measures: Specificity, sensitivity, NPV, PPV and predictive probability or C-statistic from logistical regression analyses of all AUC frequency and time points.

Results: Non-endometriosis versus endometriosis cohort interim analysis differed significantly ($p<0.001$) for median (IQR), AUC values, and percent frequency power distribution at baseline, (10, 20, and 30) minute post water-load at frequency ranges (15-20, 30-40, 40-50 and 50-60) cpm. GIMA threshold scoring revealed sensitivity and PPV of 96%, specificity and NPV of 96% and C-statistic of 100%. Ai-derived GIMA biomarkers threshold scoring predicted 25/25 subjects positive and negative for endometriosis, with surgical confirmation. Hormonal therapy, surgical stage, age nor pain score affected diagnostic accuracy.

Conclusion: EVG GIMA biomarker data with Ai-derived threshold scoring accurately distinguished participants with and without endometriosis. This interim analysis supports continued investigation of GIMA biomarkers to diagnose endometriosis.

Keywords: Biomarker; Electroviscerography; Endometriosis; Gastrointestinal Myoelectrical Activity (GIMA); Electroviscerogram; Water Load Satiety Test (WLST); AI-derived threshold score; Predictive modeling

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INTRODUCTION

Endometriosis is a chronic, complex estrogen-driven disorder, with genetic and immunologic driven variation where endometrial tissue is found in extra-uterine sites, eliciting local and systemic inflammation, fibrosis, and pain, affects 6%–10% of premenopausal women and teens, 60% of those with chronic pelvic pain, 80% of patients with dysmenorrhea, and 30%–50% of women with infertility [1-3]. Disease prevalence is likely underestimated and misdiagnosis common due to a lack of patient and health care provider awareness, normalization of dysmenorrhea symptoms, especially in teens, cultural mores around menstruation and pain in women, and symptoms that are not specific to the disease [1-2]. Accuracy of the traditional diagnostic standard, laparoscopic surgery and histopathology, is only 50% to 75% [4]. Women see multiple practitioners over 8 years-12 years until correctly diagnosed [5]. As a result, there is an average of 8.6 years from the time of symptom appearance, until final diagnosis, allowing the disease to advance exacting a toll on quality of life while disrupting educational, career goals, and personal relationships [5, 6]. The economic impact is under recognized with US direct medical costs of 26 billion and lost productivity 55 billion annually [7].

Long-term risks of untreated endometriosis, including infertility, depression, and chronic diseases like ovarian cancer, cardiovascular disease, and autoimmune diseases drive the need for new methods for early diagnosis. Tests like MRI and transvaginal ultrasound are highly accurate for advanced disease which represents 10%-15% of symptomatic women; however, diagnostic accuracy is lost after surgical intervention [4]. The remaining 70% of women suffer an average of 8.5 years before being diagnosed due to a lack of low cost, accurate, non-invasive and readily available diagnostic testing. Current non-invasive testing is resource intensive and hampered by variable diagnostic accuracy and requires the acquisition, storage, transport, complex analysis and disposal of biological materials [8-13]. Concerns of reproducibility and genetic or ethnic variability have not yet been addressed. New diagnostic testing is promising and awaits multicenter randomized control trials for further validation and broader applications in assessing disease and symptom recurrence across the lifespan [14].

Concerns regarding accuracy and expense of the current diagnostic surgical standard, have led to guideline changes, suggesting non-invasive diagnostic equivalents like MRI and ultrasound [15]. The Enzian classification for non-invasive characterization of endometriosis further emphasizes the need for validated non-invasive, cost effective and accurate diagnostic testing [16].

Introduction of diagnostic biomarkers like salivary mRNA and others, which could fulfill the need, has not resulted in recommendation changes to use biomarkers attributed to reported low accuracy and lack of more extensive validation [10, 17]. A prior published study identified a new biomarker, Gastrointestinal Myoelectrical Activity (GIMA), which showed unique specificity in diagnosing endometriosis [18]. Subsequently, GIMA biomarkers detected using Electroviscerography (EVG) confirmed the original study findings, demonstrating sensitivity, specificity and C-statistic predictability of the running spectral analysis to be 100% in a small trial cohort [19].

The robust results of EVG in diagnosing endometriosis compelled the design of the current multicenter, multi-ethnic study of GIMA biomarkers with AI-derived threshold scoring, using non-invasive EVG to validate:

- Diagnostic accuracy of the unique GIMA biomarker signature of endometriosis.
- Ability to distinguish between subjects with and without disease, and
- Validation of the AI algorithm based upon the number of variables and patients required to satisfy performance thresholds to ensure high diagnostic accuracy. The current data is the interim validation of the results.

MATERIALS AND METHODS

Study design and disease overview

This non-randomized open-label prospective comparative study to investigate the detection of a novel GIMA biomarker for endometriosis by comparing participants with known and suspected endometriosis, normal asymptomatic women, and subjects with abdominal/pelvic symptoms from other diseases without a diagnosis of endometriosis was reviewed and approved by the human Investigational Review Board IRB - C.H.C.A. Woman's Hospital, L.P., 00004260, Houston, TX.

Physiological concept of disease

Thirty-one different cytokines, including Prostaglandin E2 (PGE2) and F-alpha (PGF-a) are produced by the female reproductive system. Prostaglandin half-life is <30 seconds [20]. PGF-a causes contraction of smooth muscle resulting in spasm. PGE2 promotes peristalsis in bowel, fallopian tubes and uterus, essential for egg transport, menstruation, and delivery. Endometriosis is associated with simultaneously elevated PGE2 and PGF-a in peritoneal explants, fluid, and serum disabling bowel smooth muscle control, causing high-frequency non-propulsive seizure-like activity and bowel motility patterns detected as GIMA biomarkers. The effect mimics a drug-dose-response curve [18, 21-23]. No other diseases are known to produce simultaneously elevated PGE2 and PGF-a. Studies of over 500 subjects with other gynecological, urological, and gastrointestinal diseases failed to demonstrate endometriosis-like GIMA biomarkers.

Study procedures and protocol

Participants completed history and physical examinations, standardized pain questionnaires, and Electroviscerography (EVG) with Water Load Satiety Test (WLST). Participants stratified into three cohorts. Cohort 1 asymptomatic subjects without illness, and participants with documented disease-associated abdominal pain not diagnosed as endometriosis. Cohort 2 had histologically documented endometriosis via standard or excisional biopsy without total excision at laparoscopy. Cohort 3 included participants with abdominal or pelvic discomfort, suspicious for endometriosis with planned laparoscopy.

Originally biomarker detection was performed by transnasal small bowel manometry with intrinsic disadvantages, secondary to test methods and time length requiring 24 hours [18]. Existing Electrogastrography technology was modified to detect, record, and analyze the endometriosis GIMA biomarker frequencies [24]. The rebranded EVG technology had never been studied in formal trials.

The interim analysis evaluated the first 25 patients in each of the non-endometriosis and endometriosis cohorts in order to confirm that EVG could distinguish subjects with endometriosis from those without disease. The specific analysis assessed the accuracy of the endometriosis fingerprint GIMA biomarker with regard to sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and AUC-based predictive C-statistic. Estimation of the patient number needed for AI-derived diagnostic threshold calculation would be obtained while determining the diagnostic performance threshold.

Informed consent was obtained from all subjects as a condition to study inclusion. STARD reporting guidelines were observed for study and data analysis [25].

Study population

The full study population included 154 women aged 18 or older. Subjects were selected after satisfying inclusion criteria into one of three cohorts: Cohort 1, asymptomatic subjects without signs or symptoms of endometriosis, or with documented disease-associated abdominal pain not diagnosed as endometriosis, Cohort 2, subjects with histologically documented endometriosis at laparoscopy, and Cohort 3, included participants having abdominal or pelvic discomfort, suspicious of endometriosis pending laparoscopy. Participating clinicians and EVG technicians were blinded to results.

Participants with ASA physical status classification >III, gastrointestinal tumor or ulcers, stenosis or mechanical bowel or urinary obstruction, prior gastric or pelvic surgery, or malignancy were excluded. No changes were made to existing treatments or medications.

Electroviscerogram with water load satiety test

Standardized EVGs were recorded using hand-held devices with respiratory belts to distinguish respirations from bowel contractions [19, 24]. Three silver-chloride electrodes were positioned on the abdomen. EVGSAS custom software (3CPM Company, Sparks Glencoe, MD) performed recording and data analysis of measurements of filtered percent distribution of power at (15-20, 20-30, 30-40, 40-50, and 50-60) cpm ranges during baseline and (10, 20, and 30) minutes after water load of endometriosis-specific GIMA biomarker frequency ranges (Figure 1) [18,19].

A Running Spectral Analysis (RSA) stratifying frequency over time and AUC measurements at specified frequency ranges provided visual recognition of abnormal versus normal GIMA biomarker frequencies. AUC percent frequency distribution of power was used to statistically determine sensitivity, specificity, positive and negative predictive values as well as diagnostic predictability.

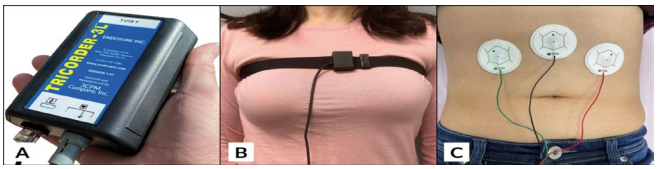


Figure 1: EVG system with A: Tricorder-3I, B: Respiratory belt and C: Dry gel electrodes.

Pain/Discomfort score

Modified ENDOPAIN 4-D standardized pain questionnaires using a 10-point verbal rating scale, recorded pain associated with menstruation, urination, sexual intercourse, defecation, abdominal and pelvic pain [26]. The calculated score was the highest single score of reported items.

Statistical methods

Baseline and clinical characteristics were compared using a Rank-sum test or Fisher's exact test for linear and binary data as applicable. p-value of 5% was used for statistical significance (Table 1).

Table 1: Comparison of baseline and clinical characteristics of cohorts.

	+Endometriosis N = 25	- Endometriosis N = 25	p-value
Age, Median (IQR)	41 (31–55)	32 (27–38)	0.01
BMI, Median (IQR)	25 (21–28)	23 (21–29)	0.94
Ethnicity, n (%)			
Asian	7 (28%)	0	0.01
Black	1 (4%)	0	
Caucasian	15 (60%)	23 (92%)	
Hispanic	2 (8%)	2 (8%)	
ENDO4D Pain Score, Median (IQR)	4.0 (3.0–6.0)	1.0 (0–2.0)	p<0.001
Pain			
No	0	6 (24%)	0.02
Yes	15 (100%)	19 (76%)	
Nausea			

No	16 (64%)	17 (68%)	p>0.95
Yes	9 (36%)	8 (32%)	
Bloating			
No	9 (36%)	16 (64%)	0.09
Yes	16 (64%)	9 (36%)	
Vomiting			
No	24 (96%)	20 (80%)	0.19
Yes	1 (4%)	5 (20%)	
Diarrhoea			
No	20 (80%)	21 (84%)	p>0.95
Yes	5 (20%)	4 (16%)	
Constipation			
No	17 (68%)	15 (60%)	0.77
Yes	8 (32%)	10 (40%)	
Reflux			
No	21 (84%)	22 (88%)	p>0.95
Yes	4 (16%)	3 (12%)	
Distention			
No	16 (64%)	18 (72%)	0.76
Yes	9 (36%)	7 (28%)	
Mode of Diagnosis -Surgery	25/25	0/25	-
ASRM Stage			
I-II	14/25	-	-
III-IV	Nov-25	-	-
Medications n (%)	10 (40)	6 (24)	
Oral Dual Contraceptives	7 (28)	6 (24)	-
Progestins	3 (12)	1 (4)	-
Androgens	1 (4)	0	-
Medicated IUD	0	1 (4)	-
GNRH agents	1 (4)	0	-
Medical Diagnoses Non-endo n (%)			
No Abnormality	0	13 (26)	-
Collagen vascular	2 (4)	1 (2)	-
Ehlers Danlos	0	1 (2)	-
Fibroids	1 (2)	3 (6)	-
Interstitial Cystitis	4 (8)	1 (2)	-
Microscopic/Ulcerative Colitis	0	2 (4)	-
PCOS	3 (6)	1 (2)	-
Polycythemia Vera	0	1 (2)	-
Thyroid Disease	0	2 (4)	-

Note: Medians were compared using a rank-sum test and percentages were compared using a Fischer's exact test. A p value of less than 0.05 was considered to be statistically significant.

Data at time (0, 10–20, 20–30 and 30–40) mins for each frequency Area Under the Curve (AUC) was calculated using the linear log trapezoidal rule. Figure 2 compares the distribution at different frequencies in women with and without EM. Clear differences indicate that AUC is a good measure to classify women in disease and non-disease groups (Table 2).

Table 2: Summary of GIMA biomarker AUC classification of disease and no disease.

EVG Frequency (min)	- Endometriosis	+ Endometriosis	p-value
10–15	1741.3 (1326.6–2022.9)	722.3 (230.1–1210.7)	p<0.001
15–20	404.1 (321.1–616.1)	310.0 (168.5–567.2)	p<0.001
20–30	384.0 (344.9–470.0)	226.5 (162.2–282.8)	p<0.001
30–40	152.9 (93.9–245.7)	284.0 (226.1–736.8)	p<0.001
40–50	55.6 (33.7–83.5)	358.1 (272.4–625.0)	p<0.001
50–60	40.3 (27.1–61.5)	230.1 (99.5–295.9)	p<0.001
60–70	16.1 (11.8–27.0)	112.5 (66.1–220.5)	p<0.001
70–80	9.1 (5.5–16.2)	34.7 (23.4–80.3)	p<0.001
80–90	6.1 (3.5–19.2)	25.1 (12.3–41.2)	p<0.001

Note: (Disease=Endometriosis) Area under the curve is calculated for each woman for a given frequency. Median AUCs were estimated and compared using the rank-sum test. Differences at every frequency level establish AUC is a good measure to classify women into disease and no-disease groups.

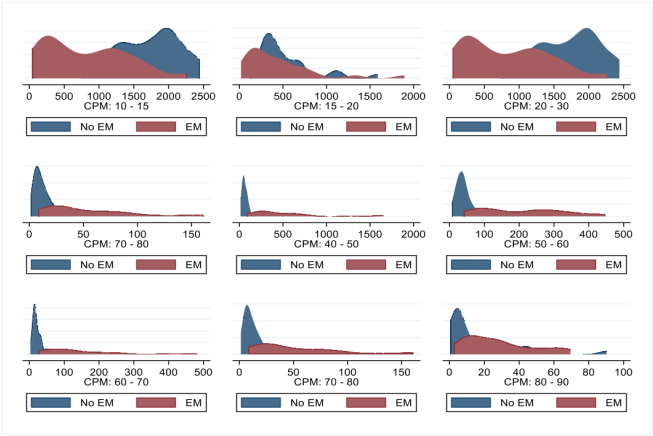


Figure 2: Distribution of AUC by different frequencies (EM=Endometriosis).

Note: Kernel plots were used to compare distribution of AUC between controls and cases for a given frequency. For all frequencies p-value was less than 0.001.

Linear regression was used to assess the relationship between calculated AUC and CPM at times (0, 10–20, 20– 30 and 30–40) mins and correlation, or agreement between estimated and calculated AUC was assessed. Since the correlation was perfect, linear regression equations were used to calculate AUC for future data points. Logistic regression assessed relationships between EM and estimated AUC for each frequency. Using this, predicted probability of EM was estimated. If the predicted probability was >0.5, women were identified as having EM. Sensitivity, Specificity, PPV, NPV, and C-statistic (Area Under the ROC Curve) was calculated for different scenarios (Table 3).

Table 3: AI-Derived permutations of prediction modelling.

	Sensitivity	Specificity	PPV	NPV	C-Statistic	Correctly Classified
AUC_{15-20}	88%	96%	96%	89%	98%	92%
AUC_{15-20} + GI Symptom Score	96%	96%	96%	96%	99%	96%
AUC_{15-20} + GI Symptom Score + Age	100%	100%	100%	100%	100%	100%
AUC_{30-40}	88%	92%	92%	88%	98%	90%
AUC_{30-40} + GI Symptom Score	100%	100%	100%	100%	100%	100%
AUC_{40-50}	68%	88%	85%	73%	89%	78%
AUC_{40-50} + GI Symptom Score	84%	92%	91%	85%	96%	88%
AUC_{40-50} + GI Symptom Score + Age	96%	96%	96%	96%	100%	96%
AUC_{15-20} + AUC_{30-40}	96%	96%	96%	96%	100%	96%

AUC_{30-40} + AUC_{40-50} + GI Symptom Score	100%	100%	100%	100%	100%	100%
AUC_{50-60}	56%	72%	67%	62%	74%	64%
AUC_{50-60} + GI Symptom Score	76%	88%	86%	79%	92%	82%
AUC_{50-60} + Age	64%	68%	67%	65%	76%	66%
AUC_{50-60}+ GI Symptom Score + Age	92%	96%	96%	92%	99%	94%
AUC_{15-20}+ AUC_{30-40}+ AUC_{40-50}+ AUC_{50-60}	**96%**	**96%**	**96%**	**96%**	**100%**	**96%**
AUC_{15-20}+ AUC_{30-40}+ AUC_{40-50}+ AUC_{50-60}+ Age	100%	100%	100%	100%	100%	100%
AUC_{15-20}+ AUC_{30-40}+ AUC_{40-50}+ AUC_{50-60}+ GI Symptom Score	100%	100%	100%	100%	100%	100%

Note: Sensitivity, Specificity, PPV, NPV, and C-statistic for different scenarios. Highlighted bold values represent pure frequency-based AI-diagnostic modelling and the most objective prediction of disease.

Raw data at each time point of GIMA biomarker frequencies was compared between non-endometriosis controls and subjects with endometriosis using a rank-sum test (Table 4).

Table 4: Distribution of GIMA biomarkers in cases and controls.

Frequency (cycles/Min)	Times of Collection	Controls (EMN) (n=25)	Cases (EM) (n=25)	p-value
10.0–15.0	Baseline	53.1 (40.5–67.7)	22.1 (85–54.3)	p<0.001
	10–20	62.2 (45.6–70.4)	21.0 (6.3–45.9)	p<0.001
	20–30	63.0 (41.7–68.3)	29.0 (7.3–36.3)	p<0.001
	30–40	58.9 (44.1–66.6)	22.2 (8.7–43.3)	p<0.001
15.0–20.0	Baseline	13.2 (9.5–23.9)	9.3 (3.2–14.8)	0.02
	10–20	14.2 (8.1–20.0)	8.7 (4.1–18.8)	0.14
	20–30	14.3 (10.3–21.8)	12.7 (6.7–21.7)	0.4
	30–40	12.6 (9.9–17.1)	9.0 (4.0–19.2)	0.14
20.0 – 30.0	Baseline	18.5 (10.6–19.1)	6.2 (4.0–1.0)	p<0.001
	10–20	12.4 (9.0–17.4)	6.8 (3.6–10.0)	p<0.001
	20–30	11.2 (10.1–15.6)	7.5 (4.8–11.5)	p<0.001
	30–40	14.4 (10.6–18.5)	9.0 (5.6–11.2)	p<0.001
30.0–40.0	Baseline	6.0 (3.7–9.9)	11.3 (4.8–21.8)	0.03

	10–20	3.9 (2.6–6.1)	8.6 (4.9–31.3)	p<0.001
	20–30	3.9 (2.4–8.1)	10.9 (7.9–23.6)	p<0.001
	30–40	5.3 (3.3–9.9)	11.6 (6.4–27.4)	p<0.001
40.0–50.0	Baseline	2.1 (1.8–3.0)	10.0 (4.3–31.7)	p<0.001
	10–20	2.1 (0.8–2.7)	15.7 (7.0–21.2)	p<0.001
	20–30	1.6 (1.0–2.7)	11.6 (5.0–18.8)	p<0.001
	30 - 40	2.0 (1.2–3.4)	9.8 (4.1–22.2)	p<0.001
50.0–60.0	Baseline	1.6 (1.2–2.4)	5.1 (3.0–7.3)	p<0.001
	10–20	1.0 (0.6–1.8)	7.0 (2.9–10.2)	p<0.001
	20–30	1.1 (0.8–1.7)	5.4 (3.7–9.1)	p<0.001
	30–40	1.5 (0.9–2.3)	6.5 (2.8–10.9)	p<0.001
60.0–70.0	Baseline	0.60 (0.55–1.16)	3.0 (1.5–6.7)	p<0.001
	10–20	0.47 (0.26–0.66)	3.5 (1.9–7.4)	p<0.001
	20–30	0.49 (0.35–0.82)	2.7 (2.1–7.3)	p<0.001
	30–40	0.54 (0.39–0.82)	3.82 (1.9–5.8)	p<0.001
70.0–80.0	Baseline	0.44 (0.21–0.77)	1.30 (0.63–2.52)	p<0.001
	10–20	0.25 (0.16–0.46)	1.11 (0.48–1.97)	p<0.001
	20–30	0.28 (0.15–0.59)	1.07 (0.65–2.10)	p<0.001
	30–40	0.24 (0.15–0.42)	1.25 (0.68–2.58	p<0.001
80.0–90.0	Baseline	0.28 (0,11–0.56)	0.78 (0.39–0.99)	0.005
	10–20	0.15 (0.07–0.28)	0.71 (0.20–1.24)	0.005
	20–30	0.22 (0.11–0.62)	0.61 (0.32–1.42)	0.006
	30–40	0.19 (0.09–1.01)	0.70 (0.39–1.38)	0.02

Note: Medians were compared using a rank-sum test and percentages were compared using a Fischer's exact test. A p value of less than 0.05 was considered to be statistically significant.

The cut off level for significance used to conduct the statistical analysis for the study was 5%. All statistical analysis utilized R 4.2.2.

Sample size for interim analysis

Considering the nature of the study, there is no applicable sample size and power calculation algorithm for the interim analysis. Instead, we calculated the power of the observed difference in the Mean AUC for different frequencies. The expectation was to use an interim AUC analysis with the initial 25 non-endometriosis controls and 25 known endometriosis subjects. The known pre-values for the frequencies being studied, already determined from a separate pilot study presented in 2022, were sufficient to

provide a greater than 80% power with a low generalizable error and to determine AUC criteria and justify completion of the study. Given the known endometriosis state of the 50 subjects, calculations suggest the power will be >80%, since the p-values are small (Table 2). Therefore, sample size was justified as more than adequate to justify the conclusions and continued evaluation of technology on a larger sample size to account for variation.

RESULTS

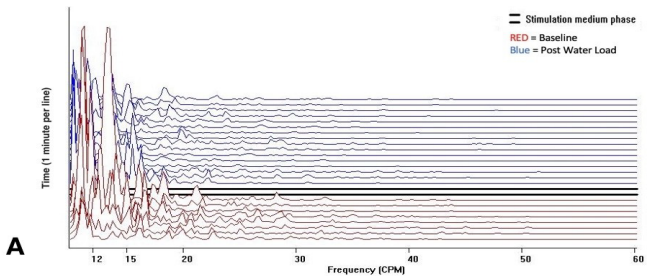
Fifty-three of the planned 150 subjects were initially recruited into the study. 27 were in Cohort 1 and 26 in Cohort 2. Three subjects, 2 (Cohort 1) and 1 (Cohort 2), failed inclusion criteria from lack of informed consent. All subjects successfully underwent EVG with WLST after completing pain questionnaires. Adequate running spectral analyses and sufficient data allowed calculation of AUC. Ai-derived GIMA threshold, scores, were calculated for each subject. Clinical and demographic characteristics of the 50 patients in the interim validation cohort are presented in table 1.

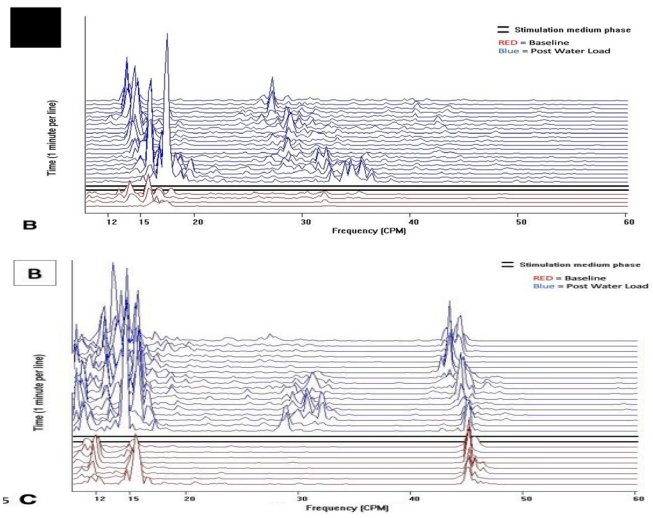
Among the 50 subjects in the interim analysis, 25 had histologically documented endometriosis and 25 were divided between 6 asymptomatic and 19 symptomatic non-endometriosis controls. Of those with endometriosis, ASRM stages 1-4 were reported to be represented equally, without significant differences. Endo4D pain scores analyzed using median IRQ, used VAS ranging from 1-10, with 10 being the highest level of pain. Pain was highly associated with EM positivity, with statistically significant differences ($p<0.001$) observed compared to the EM negative cohort.

Hormonal therapy was noted in 6 (24%) in the non-endometriosis Cohort 1 and 10 (40%) in Cohort 2. Cohort 1 hormonal treatments were: 6 (24%) combined oral contraceptive pill, 1 (4%) progestin, 1 (4%) medicated IUD and no GNRH agonist. Cohort 2 reported: 7 (28%) oral contraceptive pill, 3 (12%) progestin, 1 (4%) androgen, 0 medicated IUD and 1 (4%) GnRH agonist.

RSA – qualitative analysis

Cohort 1 qualitative visual patterns were flat without the diagnostic 15 cpm-60 cpm GIMA biomarker pattern. (Figure 3a). Comparatively, cohort 2 showed endometriosis-associated increased GIMA biomarker activity at (15-20, 30-40, and 40-50) cpm frequency ranges (Figures 3b and 3c). These differences, noted in 25/25 of Cohort 2 subjects, were absent in Cohort 1 subjects. These qualitative findings confirmed histopathological positivity and/or absence of EM.





Figures 3a-c: Running spectral analysis of Cohort 1-3.

Note: Power of frequency distribution of GIMA characteristics at frequencies (10 cpm-60 cpm) among A) non-endometriosis participants, who were with or without symptoms at baseline and 10 min, 20 min, and 30 min post water load, B) among subjects with endometriosis Cohort 2, and C) among subjects with endometriosis Cohort 3.

EVG GIMA biomarker predictive modeling

EVG derived GIMA biomarker percent frequency distribution of power for median (IQR) between Cohort 1 and EM-positive Cohort 2 were significantly different ($p<0.001$), for frequencies 20 cpm-90 cpm at baseline, and (10, 20, and 30) minutes post water load (Table 4). Subjects with histologically confirmed endometriosis had the same quantitative GIMA biomarker positivity. AUC calculated for all GIMA biomarker frequency cycles was significantly higher ($p<0.001$) for cohort 2 versus control cohort 1. Higher GIMA biomarker AUC values were highly associated with EM, demonstrating GIMA biomarker diagnostic specificity and predictability (Figure 2 and Table 2). Data validates that non-invasive GIMA biomarkers can predict or exclude EM in asymptomatic and symptomatic women with suspected EM. Kernel plots of individual frequencies in figure 2, comparing distribution of AUC between EM+ cases and EM-controls per frequency, show the dominant ability of GIMA biomarkers to distinguish disease from disease states. For all frequencies $p<0.001$was noted.

EVG derived GIMA biomarker algorithm for predicting endometriosis

The AUC of all GIMA biomarker frequencies were measured and calculated at baseline, (10, 20, and 30) minutes. Multivariable logistic regression models assessed the effect of AUC GIMA biomarker frequencies and associated age and symptom score variables. Ai methods determined which model had highest C-Statistic values and lowest misclassification rate to estimate disease probability. Predicted probability of EM, calculated from logistic regression equations, was positive if >50%. This supervised predictive model diagnosed EM when probability threshold value was ≥ 0.5 and predicted negativity with threshold under 0.5.

GIMA biomarker model performance

The objective model displayed 96% sensitivity, 96% specificity, 96%, Positive Predictive Value (PPV), 96% Negative Predictive Value (NPV) and a 100% C-statistic in women with disease (Table 3). Using the Ai-derived model to predict disease in this interim analysis, area under the ROC curve is calculated to be 0.9984, for the AUC15-20+ AUC30-40+ AUC40-50+ AUC50-60 (Figure 4). The closer the ROC to 1.0, the higher the predictive value of the test which is in line with the calculated c-statistic. The data used to calculate model performance was entirely objective, when excluding subjective data such as pain score.

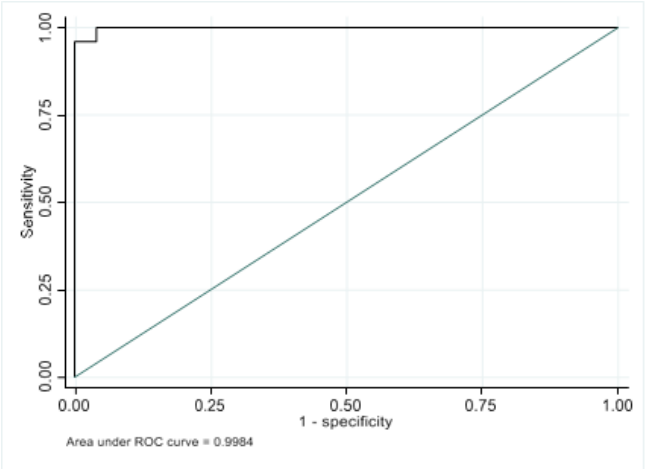


Figure 4: ROC curve for Ai derived model scenario AUC15 -20+ AUC30-40+ AUC40-50+ AUC50-60.

Note: Area under the ROC curve is 0.9984. The closer to a value of 1.0 the higher the predictive value of the test.

DISCUSSION

Main findings

This interim analysis, part of a larger prospective multicenter validation trial, confirms the diagnostic capabilities, accuracy, and performance of EVG technology to detect the GIMA biomarker unique to endometriosis. The stability and reproducibility of GIMA biomarkers coupled with Ai-derived diagnostic threshold levels to detect endometriosis versus non-disease status is confirmed by interim data. In addition, the accuracy regardless of ethnicity suggests potential applicability across international populations. The two centers represent two different medical models spanning from the expert tertiary care center to the routine outpatient setting, with differing levels of severity of disease presentation.

Specifically, the interim data showed the ability to detect and differentiate between endometriosis confirmed subjects and non-endometriosis controls, with 96% sensitivity and PPV, 96% specificity and NPV, and 100% C-statistic predictability, regardless of age or pain scores (Table 3).

Biomarker activity unique to endometriosis is driven by PGE2 and PGFα mediated 15 cpm-60 cpm smooth muscle GIMA activity. Age, concurrent hormonal therapy, or stage of disease had no impact, nor did confounding illnesses like inflammatory bowel diseases, irritable bowel syndrome, urinary or pelvic infection, chronic interstitial cystitis, biliary or ulcer disease, and polycystic ovary disease. The endometriosis-free cohort age differed significantly with the endometriosis positive cohort, but the strength of analysis suggests this did not influence the results.

Statistical modeling

Statistical and predictive modeling used in the interim analysis is essential and critical. The model was chosen to duplicate modeling conditions for the larger cohort. It consisted of Ai-derived modeling based upon multiple variables including AUC, pain scores and age, which demonstrated model strength despite smaller number of patients in the interim analysis. Given model strength and accuracy exceeding 98%, it is applicable to the final intended study population of over 150 patients. Predictive modeling is a process in evolution, expected to achieve increasing precision with inclusion of larger populations. Results of the interim data were noted in two disparate populations with largely known rates of occurrence or absence of disease. Final validation will come from the complete study which includes a large validation cohort of undiagnosed symptomatic subjects, and additional disease-confirmed and disease-free participants.

Strengths and limitations

The EVG device resembles devices including the handheld electrocardiogram making it intuitive, recognizable, and practical with procedural proficiency after 1-2 procedures [19, 24]. Low procedural costs and minimal technical proficiency required to perform the test will translate into greater availability.

Nonetheless, inherent limitations should be recognized. Firstly, studies performed in ideal or tertiary research settings may not translate directly into real-world clinical practice and further testing is needed in larger varied populations of patients. The interim analysis data is compelling but requires confirmation by the power of the full study, especially the validation cohort of symptomatic undiagnosed women. Secondly, with studies including control groups not suspected of having endometriosis, finding ideal disease-free subjects is inherently difficult, with many ways to miss endometriosis especially in asymptomatic women and with a recognized surgical diagnostic accuracy of only 50%-75% [27]. In this particular study population's control group of this interim analysis, it was encouraging to see the homogeneity of absent GIMA biomarkers compared to subjects with known disease [4].

While the data is statistically sufficient to justify conclusions, model-building is an evolving process. Continued experience will refine the model, account for potential population variances, improve predictability, confirm GIMA biomarker threshold score variations, predict surgical stage/disease activity, facilitate post-treatment monitoring, and assess hormonal suppression impact. Hormonal suppression did not affect study results, yet newer suppressants may exert unknown effects. Other coexisting disease may simultaneously secrete PGE2 and PGFα, presenting as false positive results. Adenomyosis could have similar GIMA biomarkers impacting the GIMA biomarker threshold scoring. Finally, the interim analysis it is not powered to

make definitive conclusions regarding confounding effects of ethnic variation, the presence of other conditions such as uterine fibroids, ovarian disease, benign gastrointestinal or urological disease, and inflammatory conditions of the digestive, gynecological and urological systems. This will require a wider clinical study of larger affected patient cohorts.

Interpretation

The future application of this technology will depend on the severity and presentation of disease. Advanced stage disease presents in 15% of women and is easily diagnosed via transvaginal or trans anal ultrasound, MRI, or physical exam [9]. In this subpopulation, GIMA biomarker detection will find greatest application in providing long-sought-after post-therapeutic monitoring, which with current imaging technology is limited by diagnostic differentiation of postoperative changes versus recurrent disease. Most compelling is the 70% of women with early-stage disease who remain symptomatic, suffering from delayed diagnosis or inadequate response to medical therapy. GIMA biomarker threshold scoring could reduce typical diagnostic delays in undiagnosed women waiting for decades with an average of 8.3 years between symptom onset and diagnosis in patients with pain, contrasted with 1.8 years in those with infertility [28, 29]. For the 10%-15% of women who are asymptomatic and present only with infertility, GIMA biomarker testing may play a central diagnostic role, especially given recent restrictions on diagnostic laparoscopy in this group. Non-invasive testing plays an equally important role when cultural complexities, sexual discrimination, and geographic and financial barriers influence delay [30].

Currently, non-invasive diagnostic options are either lacking, expensive, has limited availability or diagnostic accuracy [8]. Transvaginal ultrasound and MRI are effective but limited in early disease or following surgical treatment [9]. Blood mRNA or mutated DNA elements have varied accuracy, requiring biological material handling and laboratory services [10, 11]. Menstrual fluid and endometrial scrapings face similar challenges [12, 13]. The more recent use of salivary mRNA shows promise and despite reported sensitivity and specificity of 95% to 100%, AUC diagnostic accuracy is variable ranging from 69%-98%, with further challenges due to logistics and high cost [10, 31, 32]. In contrast, GIMA biomarker testing is not hampered by logistical challenges of biological sampling, laboratory infrastructure, specialized facilities, transportation and cost.

As with any diagnostic test being introduced into routine clinical practice, significant external validation is required to answer necessary questions concerning specificity and applicability across broad, diverse ethnic populations with variable disease. However, once accomplished, immediate clinical benefits are timely non-invasive diagnosis, earlier access to therapy for symptom control, and limitation of disease progression. No guarantees exist regarding disease advancement, as randomized clinical trial data, with laparoscopy before and after an interval without treatment, demonstrated 30% short-interval advancement, without means of predicting progression [33, 34].

Low cost, accurate, and mobile testing would decentralize access, allowing universal rapid deployment, shortening time between initial presentation, diagnosis, and treatment. With reproducibility in general populations, a positive impact on participants, caregivers, medical costs, and productivity is expected, eliminating unintended geographical or financial discrimination [35].

CONCLUSION

This interim analysis of a prospective multicenter study provides data on the diagnostic accuracy of the unique GIMA biomarker to distinguish between subjects with or without disease. Combining EVG biomarker detection with AI-derived threshold scoring demonstrated a non-invasive tool with beyond reasonable accuracy to determine a diagnosis of endometriosis. With further validation in the larger planned validation study cohort and other confirmatory studies, it is reasonable to expect this new non-invasive test will overcome the natural skepticism of novel methodologies, and existent unintentional geographic and financial barriers insuring timelier diagnosis of this devastating disease.

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DISCLOSURE OF INTEREST

MN reports a relationship with Endosure, Inc as a founder and board member. MN has patent #7,160,254 licensed to Endosure, Inc. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CONTRIBUTION TO AUTHORSHIP

Conceptualization, Mark Noar and John Mathias; Data curation, Mark Noar and Ajit Kolatkar; Formal analysis, Mark Noar and Ajit Kolatkar; Investigation, Mark Noar and John Mathias; Methodology, Mark Noar and John Mathias; Project administration, Mark Noar, John Mathias and Ajit Kolatkar; Resources, Mark Noar, John Mathias and Ajit Kolatkar; Software, Mark Noar; Supervision, Mark Noar and Ajit Kolatkar; Validation, Mark Noar and Ajit Kolatkar; Visualization, Mark Noar and John Mathias; Writing – original draft, Mark Noar; Writing – review & editing, Mark Noar, John Mathias and Ajit Kolatkar.

DETAILS OF ETHICS APPROVAL

The procedures of the study received ethics approval form the Institutional ethics committee, and complied with the required regulations governing human study as per the Declaration of Helsinki. The name of the IRB was the human Investigational Review Board IRB - C.H.C.A. Woman's Hospital, L.P., Houston, that approved the study June 23, 2007, reference number 00004260.

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