Diagnostic Accuracy of Urinary Cytokeratin 19 Fragment for Endometriosis

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Abstract

Endometriosis affects up to 10% of women of reproductive age and 176 million women worldwide. The prevalence in women with infertility is between 30% and 50% but may be higher in women with pelvic pain, interstitial cystitis, or irritable bowel syndrome. Cytokeratin 19 has been suggested as a potential biomarker in urine for the diagnosis of this condition. The objective of this study was to prospectively determine the accuracy and the performance of a urinary cytokeratin 19 (uCYFRA 21-1) test for diagnosing endometriosis. Ninety-eight consecutive women who underwent laparoscopy had a urinary sample obtained before surgery and were included in the study. Endometriosis was diagnosed by laparoscopy and pathology in 64.3% (63 of 98 women). The estimates and 95% confidence intervals for sensitivity, specificity, positive and negative predictive values, and likelihood ratios were 11.1% (4.5%-21.5%), 94.3% (80.8%-99.3%), 77.7% (39.9-97.1), 37% (27-47.9), 1.94 (0.43-8.86), and 0.94 (0.84-1.06), respectively. Despite the high specificity, the uCYFRA 21-1 test has limited value for clinical practice to discriminate between women with and without endometriosis.

Keywords

endometriosis, sensitivity, specificity, cytokeratin 19

Introduction

Endometriosis is defined as the presence of endometrial tissue implants outside the uterus. This condition affects approximately 10% of women of reproductive age¹ while the prevalence of women with endometriosis who also have infertility or pelvic pain is between 30% and 50% and 70%, respectively.² The symptoms of women with endometriosis are highly variable ranging from minimal to acute abdominal pain.³ Endometriosis is often undiagnosed, or the diagnosis is delayed for up to 12 years.⁴ In addition, a large cohort of women with seemingly unrelated conditions such as irritable bowel syndrome or interstitial cystitis has endometriosis that may never be attributed to this disease.^{5,6}

The current gold standard method for diagnosing endometriosis is laparoscopy, but surgery can be expensive and requires a general anesthesia, contributing to the delay in a definitive discovery of endometriosis. Likewise, the role of laparoscopy with the advance of in vitro fertilization has been questioned.⁷ Nevertheless, women with even mild endometriosis can fail in vitro fertilization only later to be diagnosed and successfully treated.⁸ In addition, visual diagnosis of endometriosis without histological conformation has been found to be unreliable.^{9,10}

The use of biomarkers for diagnosis of endometriosis is attractive and would greatly facilitate the management of infertility and pelvic pain. Many have pursued a clinical test based

on endometrial biopsies, peripheral blood, or in other bodily fluids but success has been limited.¹¹ More recently, researchers in Australia reported the use of urinary cytokeratin 19 as a potential biomarker of endometriosis with high specificity and sensitivity in a limited study population.¹² The objective of this study was to estimate the diagnostic performance of the urinary cytokeratin 19 fragments test for endometriosis in a prospective study design, using a population similar to that found in clinical practice. Given that the performance of a screening test depends greatly on the prevalence of the disease in the studied population,¹³ we used a hypothetical range of prevalence for endometriosis to assess the performance of this potential diagnostic test in different settings.

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Methods

Participants

A consecutive series of women submitting to laparoscopy were invited to participate in a prospective study at the Greenville Health System/University Medical Center in Greenville, South Carolina, between January and April 2011. The inclusion criteria consisted of women between 18 and 50 years old presenting for a laparoscopic procedure for either tubal ligation, chronic pelvic pain, or infertility workup. Those who refused to participate were excluded. Demographic factors and menstrual history were recorded before a urine sample was taken. The institutional review board from Greenville Hospital System approved the study protocol, and written informed consent was obtained from all patients.

Measurement of Cytokeratin 19 (Cyfra 21-1) Fragment in Urine (Index Test)

A urine sample was collected upon enrollment and prior to the laparoscopic procedure. The urine was stored on ice and/or at a refrigerated temperature for no more than 2 hours prior to processing. Samples were centrifuged (400g for 5 minutes), aliquoted, and kept frozen at -70°C until analysis within 48 hours. Testing was performed at Abbott Laboratories (Abbott Park, Illinois) by a trained technician blinded to the results of the laparoscopic procedure. Samples were thawed for a minimum of 1 hour at room temperature and centrifuged at 1500 rpm for 5 minutes and tested in a single day using the CYFRA 21-1 research only assay developed for a urine application (uCYFRA 21-1) on Abbott's ARCHITECT i2000SR analyzer. The uCYFRA 21-1 assay is a 2-step immunoassay for the quantitative determination of cytokeratin 19 fragments, or CYFRA 21-1 antigen, in human urine using chemiluminescent microparticle immunoassay technology. In the first step, sample, anti-CYFRA 21-1 (KS 19.1)-coated paramagnetic microparticles, and uCYFRA 21-1 assay diluent are combined for 18 minutes. After washing, anti-CYFRA 21-1 acridinium-labeled conjugate (BM 19.21 Ab) was added to the reaction mixture and incubated for 4 minutes. Following another wash cycle, pretrigger and trigger solutions were added to the reaction mixture. The resulting chemiluminescent signal was measured as relative light units (RLUs). A direct relationship exists between the amount of CYFRA 21-1 antigen in the urine sample and the RLUs detected by the ARCHITECT iSystem optics. In addition, a urine chemistry panel (creatinine and protein) was also measured on the ARCHITECT c8000 instrument using commercially available ARCHITECT reagents. The uCYFRA 21-1 assay was tested in duplicate to check reproducibility and was normalized by urinary creatinine or protein. The inter- and intra-assay variability of uCYFRA 21-1 was less than 5%. Reportable range was 0.5 to 100 ng/mL based on a functional sensitivity of 0.5 ng/mL with a concentration of coefficient of variability of 10%; total precision with a variation of less than 5%.

Table 1. Demographic Data of Women Recruited for uCYFRA Evaluation.

Parameters	Controls (n = 35)	Endometriosis (n = 63)	P
Age, median (range)	36 (21-45)	32 (18-44)	.01 ^a
Stage of endometriosis, n (%)			
I	na	31 (49.2)	
II		19 (30.2)	
III		9 (14.3)	
IV		4 (6.3)	

Abbreviation: na, not applicable.
^aMann-Whitney test.

Reference Test for Endometriosis—Laparoscopy

Suspected positive endometriotic lesions were biopsied during laparoscopy and confirmed by pathology. Staging of endometriosis was assigned according to the Revised American Society for Reproductive Medicine score (stages I-IV).¹⁴ Only cases with pathological confirmation were included.

Statistical Methods and Sample Size

Data were analyzed using receiver–operating characteristic (ROC) curve analysis in order to identify the best cutoff for sensitivity and specificity, if the area under the curve (AUC) was >0.5 . The best cutoff was identified by the highest specificity value. Next, the same data were analyzed using a contingency table for diagnostic test with 95% confidence intervals (95% CIs). GraphPad Prism v.6 for Mac (GraphPad Software, La Jolla, California) was used for the statistical analysis. Sample size was calculated according to the literature, considering a prevalence of endometriosis of $45\% \pm 5\%$, a specificity and sensitivity of $93\% \pm 7\%$, that is, from 87% to 100%.¹⁵ The analysis yielded a minimum of 93 cases.

Results

Demographic and Clinical Characteristics

Data collection was done between January 1 and April 30, 2011. Urine for the index test (uCYFRA 21-1) was obtained within 1 hour before the laparoscopy (reference standard). A total of 98 patients were obtained for the study. No complications were reported in the patients submitted to laparoscopy or the uCYFRA 21-1 test. Clinical and demographic characteristics of the studied population are depicted in Table 1. No significant differences were found in urinary mean ($\pm$ standard deviation) levels of creatinine (132.8 ± 91.85 vs 144.1 ± 93.58) and of protein (7.4 ± 5.50 vs 13.1 ± 22.64) in patients with and without endometriosis, respectively. The prevalence of endometriosis in the studied population was 64.3%. As expected, the urine test did not yield any complications in women who underwent laparoscopy. The inter- and intra-assay variability of uCYFRA 21-1 assay was $<5\%$.

Figure 1. A, Analysis of the expression of urinary cytokeratin 19 fragments (uCYFRA) in women with and without endometriosis. No significant difference was found (Mann-Whitney *U* test). B, Receiver–operating characteristics (ROC) of uCYFRA for the diagnosis of endometriosis. The area under the curve (AUC) reveals the low performance of the test. The highest specificity was obtained with a cutoff of 5.3 ng/mL (dotted line in A).

Figure 2. Flow chart of the study using a 5.3-ng/mL cutoff.

Receiver–Operating Characteristic Curve and Definition of Cutoff

The median values of uCYFRA 21-1 were not significantly different between groups ($P = .59$; Figure 1A). The AUC for the 98 specimens for uCYFRA 21-1 was 0.53 (Figure 1B). Normalizing the uCYFRA 21-1 results using urine creatinine or urine

protein resulted in AUC values of 0.54 and 0.57, respectively (data not shown). From the ROC curve, the 5.3 ng/mL cutoff was chosen for yielding the highest specificity (94.3%, 95% CI 80.8–99.3). With this cutoff, 2 (5.71%) of the 35 patients had a false-positive test (Figure 2). The performance of the test with this cutoff revealed an accuracy of 40.8% (95% CI 31.6–50.7)

Table 2. Performance of the uCYFRA Test (n = 98) With 5.3 ng/mL as the Cutoff.

Parameter	Estimate	95% Confidence Interval
Sensitivity	11.1%	4.5-21.5
Specificity	94.3%	80.8-99.3
Positive predictive value	77.8%	39.9-97.1
Negative predictive value	37.1%	27.0-47.9
Accuracy	40.8%	31.6-50.7
Positive likelihood ratio	1.94	0.43-8.86
Negative likelihood ratio	0.94	0.84-1.06

and a positive and negative likelihood ratio (LR) of 1.94 and 0.94, respectively (Table 2). Similar results were observed if different stages of endometriosis were considered (data not shown).

Discussion

When a premenopausal woman consults with her gynecologist with a complaint of chronic pelvic pain or intense dysmenorrhea, her physician will likely consider that the odds of her having endometriosis is high based on her symptoms. Due to the benign course of asymptomatic endometriosis, clinicians may have less interest in a screening test to confirm the condition, than other diseases. Further, actions based on a positive test require laparoscopy with histological confirmation, which can be both costly and invasive. Therefore, a desirable test for endometriosis would have a low false-positive rate that is used to calculate positive predictive value and specificity of a test.¹³ The level of CYFRA 21-1 in urine has a high specificity (94.3%), but a very low sensitivity (11.1%), which is reflected by an AUC of 0.53 (Figure 1B). However, from a clinical point of view, sensitivity and specificity are by themselves of little use. The positive and negative predictive values of a test are more intuitive for a clinician.¹³ The uCYFRA 21-1 has a reasonable positive predictive value (77.8%) but an unacceptably low negative predictive value (37.1%).

At first glance, a high specificity (94.2%) and positive predictive value of 0.777 for uCYFRA 21-1 might seem to have clinical value to confirm endometriosis; when the uCYFRA 21-1 is positive, a laparoscopy will confirm the diagnosis in approximately 78% of the cases. Nevertheless, predictive values are influenced by the prevalence of the disease.¹⁶ The LR is a better approach to interpret the results of a test, as long as the prevalence of endometriosis is known in the clinical setting. For instance, using the LR obtained from Table 2, we provide a range of hypothetical prevalences of endometriosis (10%, 40%, 60%, and 80%) and the interpretations of a positive or negative uCYFRA 21-1 test results (Table 3). Despite the high specificity, the uCYFRA 21-1 test has limited clinical value, either as a positive or as a negative test. Similar performance is expected with different cutoffs. The nonsignificant differences between the distributions (Figure 1A) and the non-informative AUC (Figure 1B) reveal the low practicability of

Table 3. Probability to Have or Not Have Endometriosis (Posttest Probability) With a Positive or Negative uCYFRA Test Result, Using a Cutoff of 5.3 ng/mL.

Prevalence (Pretest Probability)	Probability of Having Endometriosis When the Test Is	
	Positive, %	Negative, %
10%	17.8	9.5
40%	56.5	38.6
60%	74.5	58.6
80%	88.6	79.0

the test in the clinical setting. In summary, in this diagnostic test trial, we demonstrate that uCYFRA 21-1 has limited clinical value as a biomarker for endometriosis.

Authors' Note

The work reported was done at Department of Obstetrics and Gynecology, Greenville Health System, Greenville, SC, USA and Diagnostic Division, Abbott Laboratories, Abbott Park, IL, USA.

Declaration of Conflicting Interests

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