Diagnostic accuracy of Cancer Antigen 125 (CA125) for endometriosis in symptomatic women: a multi-center study.

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Condensation

Raised serum Cancer Antigen 125 is a highly specific, rule-in, test for endometriosis amongst women with pain or subfertility.

Abstract

Study Objective: To assess the diagnostic accuracy of serum Cancer Antigen 125 (CA 125) ≥ 30 units/milliliter (u/ml) for diagnosing endometriosis in symptomatic women.

Study Design: Prospective observational cohort study including patients with symptoms of pelvic pain or subfertility undergoing elective diagnostic laparoscopy at two tertiary referral hospitals. We excluded patients suspected to have other gynecological pathology. We evaluated the accuracy of serum CA 125 (index test) with histologically confirmed endometriosis (reference standard).

Main Results: Fifty-eight consecutive women recruited between October 2013 to March 2015. Women with endometriosis had a higher CA 125 level than those without endometriosis (mean 54.7 +/-71.6 vs 16.2 +/- 8.0). The specificity of CA 125 ≥ 30 u/ml was 96% (95% CI 81.7 – 99.9%) and sensitivity was 57% (95% CI 37.4 – 74.5%). The positive likelihood ratio for the histological presence of endometriosis with a CA 125 ≥ 30 u/ml was 15.8 (95% CI 2.3-112) providing a post-test probability of 94% (95% CI 71% - 99%) in women with pelvic pain or subfertility. The area under the curve, 0.85 (95% CI 0.74 – 0.96) indicates high test accuracy.

Conclusions: CA 125 ≥ 30 u/ml is highly predictive of endometriosis in women with symptoms of pain and / or subfertility. CA 125 should be considered as a rule-in test for expediting the diagnosis and management of endometriosis, CA 125 <30 u/ml is, however, unable to rule out endometriosis.

Keywords: Endometriosis, Diagnosis, Cancer Antigen 125.

Introduction

Endometriosis is defined as the presence of functional endometrial like glands and stroma located outside the uterus. It is a disease clinically characterized by pain and associated with subfertility. The prevalence of endometriosis is estimated to be 10% in reproductive age women and up to 75% of symptomatic women [1]. The gold standard diagnostic test is visualization, biopsy and histological confirmation. Evaluation of non-invasive diagnostic biomarkers has not identified an accurate non-invasive test for the detection of endometriosis [2,3]. The development of a non-invasive rule in test for endometriosis could reduce time to diagnosis, provide psychological reassurance, offer treatment options, and reduce disease progression through earlier recourse to treatment [4]. Cancer Antigen 125 (CA 125), a well-established marker for epithelial cell ovarian cancer, is derived from coelomic epithelia including the endometrium, fallopian tube, ovary, and peritoneum [5]. CA 125 is raised in endometriosis through stimulation of coelomic epithelia [6]. Previous diagnostic accuracy studies have suffered from

verification bias (visual diagnosis), design bias (case-control) or clinical heterogeneity (additional gynecological disease) [1].

We performed a prospective cohort study to evaluate the diagnostic accuracy of serum CA 125 for the presence of histologically confirmed endometriosis.

Methods

The regional and local ethics committee approval was sought for the study protocol. This study was conducted as a prospective observational cohort study (Research Ethics Committee reference number: 10/H0711/24). The cohort included women with pain symptoms and or subfertility undergoing laparoscopic investigation. We report the findings in accordance with the Standards for Reporting Diagnostic accuracy studies (STARD) [7]

Patient selection and data collection

All included participants signed a written informed consent form. The study recruited participants consecutively between October 2013 – March 2015. We prospectively collected clinical data from women referred for investigation of gynaecological pain symptoms and or subfertility cared for at The Royal London Hospital, London and St Bartholomew's Hospital, London. Both institutions are British Society for Gynaecological Endoscopy (BSGE) approved endometriosis centers with experience of diagnosing and managing women with endometriosis. The BSGE endometriosis centers need to fulfil a

number of requirements including working in appropriate multidisciplinary clinical teams, auditing their outcomes and having sufficient workload to maintain their surgical skills [8].

Dysmenorrhea, dyspareunia or chronic pelvic pain was measured using visual analogue scales (VAS) 0-10cm. This was chosen as it was the most frequently reported pain outcome measure in endometriosis trials [9]. The definition used for subfertility was unexplained failed conception after 12 months of regular unprotected vaginal intercourse [10]. Patients were excluded if they were believed to have or previously had a condition other than endometriosis which can cause a raised CA 125. These conditions included previous or suspected; leiomyoma, adenomyosis, pelvic inflammatory disease (PID), mature cystic teratoma, mucinous cystadenoma, and hydrosalpinges. These were evaluated with medical history and either ultrasound scan (USS) or magnetic resonance imaging (MRI). Women with a history of any malignancy or those who did not consent were excluded from analysis.

Participants were recruited and consented prior to surgery. Serum samples were collected preoperatively for CA 125 immunoassay measurement (Roche Diagnostics, Indianapolis, United States of America). The participants underwent routine operative surgical management of endometriosis from a consultant gynecologist on the same day. The surgeons performing the procedures were blinded to the result of the CA 125 test that was processed in a certified laboratory within 4 hours of sampling with an automated immunoassay. Laparoscopy was performed and all recognizable

endometriosis lesions were biopsied and then treated by either coagulation, excision, or ovarian cystectomy. In accordance with ESHRE guidance [11], histological confirmation of disease was attempted but not possible in all cases of suspected endometriosis. As the diagnosis of endometriosis has poor accuracy based on visual diagnosis alone [12], the authors decided to exclude those participants without histological confirmation of disease a priori. Those patients with visually confirmed endometriosis or other pelvic pathology at the time of surgery were excluded from the primary analysis.

Data were collected during face-to-face interviews with each patient by a single researcher (MH) in the preoperative assessment area. We collected general information for all participants including age, gravidity, parity, age at menarche, stage of menstrual cycle, smear history, previous surgery, medication, infertility duration, smoking status, alcohol status, and contraceptive use. Gynecological pain symptoms were assess using VAS 0-10cm for dysmenorrhea, deep dyspareunia, and chronic pelvic pain, dyschezia, and dysuria. We did not control for the following confounders: hormonal use or stage of menstrual cycle.

The primary outcome was the diagnostic accuracy of CA 125 ≥ 30 u/ml to detect the presence of histologically confirmed endometriosis. Secondary outcomes included evaluating the gynecological pain symptoms between those with and without endometriosis.

Statistical analysis

Statistical data were collected in a computerized database and analyzed by SPSS software 18.0.0 (SPSS Inc., Chicago, Illinois). We compared the clinical characteristics between those with endometriosis and those without, summarizing the characteristics of the two groups using standard statistics. These two groups were classified as either reference standard (histological endometriosis) positive or negative. We then calculated the area under the receiver operating curve (ROC), which quantifies the ability of the index test (CA 125) to distinguish between patients with and without endometriosis. Our sample size was chosen so that if the true AUC was 0.85, we would be able to estimate it to within 0.15 using a 95% CI [13]. Positive likelihood ratios and negative likelihood ratios were calculated and post-test probability was evaluated using these likelihood ratios and Fagan's Nomogram [14] based on a pre-test prevalence estimate of 50% in this group of symptomatic women [15].

Results

Primary study

A total of 141 consecutive participants undergoing laparoscopy were approached for recruitment. 102 participants met the previously described inclusion criteria with sub-fertility and or gynecological pain symptoms. We prospectively recruited 67 women without evidence of previous fibroids, ovarian cysts (other than endometrioma), PID, adenomyosis, or hydrosalpinges. Nine patients were excluded at the time of surgery:

biopsy of suspected lesions was not possible (n=7); additional disease was noted (n=1); and failed laparoscopic entry (=1). One study participant who did not undergo the procedure due to failed laparoscopic entry secondary to insufflation of the pre-peritoneal space. This prohibited safe primary trocar insertion. The patient was observed overnight and followed up in clinic without complication. Fifty-eight women were included in the primary analysis (figure 1). Of those included, 28 had no macroscopic pathology and 30 were found to have histologically confirmed endometriosis (figure 1).

Clinical characteristics

We excluded 84 participants for the following reasons; suspected adenomyosis, suspected leiomyoma, previous pelvic inflammatory disease or sexually transmitted infection, previous malignancy, and visually suspected endometriosis. A total off 58 participants were included for analysis. The clinical characteristics of the participants are summarized in table 1. At the time of surgery, endometriosis was staged according the revised American Fertility Scoring for endometriosis and were classified as follows: 7 stage 1, 9 stage 2, 10 stage 3, 4 stage 4 [16]. Twenty-six participants were recruited during the follicular phase and 22 during the luteal phase of the menstrual cycle. We were unable to determine the phase of the menstrual in 10 participants due to hormonal contraceptive use.

Primary results

The mean age for those with confirmed endometriosis was 34.1 (SD +/- 5.9) and without endometriosis 32.2 (SD +/- 8.6).

Mean CA 125 values

Thirty participants diagnosed with endometriosis had a mean CA 125 level of 54.7 u/ml (SD 71.6). Twenty-eight participants with no macroscopic pathology had a mean CA 125 of 16.2 u/ml (SD 8.0). One patient had a CA 125 ≥ 30 u/ml without macroscopic gynecological disease while 17 had both a CA 125 ≥ 30 u/ml and histological endometriosis. Thirteen participants had a CA 125 < 30 u/ml in the presence of histological endometriosis while 27 had a CA 125 < 30 u/ml in the absence of macroscopic endometriosis.

Diagnostic accuracy

Receiver operating characteristic curve (figure 2) demonstrates the accuracy of CA 125 ≥ 30 u/ml as a diagnostic test for endometriosis. The area under the curve, 0.85 (CI 0.74 – 0.96) indicates high test accuracy. The use of a predefined cut-off, CA 125 ≥ 30 u/ml is based on a previously published meta-analysis [1]. This will enable further data-synthesis in the future with a comparable cut off. The chosen cut-off value (30 u/ml) demonstrated 57% (95% CI 37.4 – 74.5%) sensitivity, 96% (95% CI 81.7 – 99.9%) specificity, and 76% diagnostic accuracy. The positive likelihood ratio is 15.8 (2.3-112) providing a high positive post-test probability of 94% amongst symptomatic women with CA 125 ≥ 30 u/ml. The negative likelihood ratio is 0.45 [95% CI 0.30-0.68]

producing a negative post-test probability of 33% in women with CA < 30 u/ml and common gynaecological symptoms.

Secondary results

Pain symptoms

We compared pain symptoms between those with endometriosis and those without endometriosis. Individual patient values were combined to produce means with standard deviations (SD) calculated. The mean VAS for dysmenorrhea amongst those women with endometriosis was 8.10cm (SD 1.41cm), and for women without endometriosis was 6.49cm (SD 2.97cm). The mean VAS for dyspareunia amongst those women with endometriosis was 5.26cm (SD 3.31cm), and for women without endometriosis was 4.53cm (SD 3.79cm). The mean VAS for chronic, non-cyclical pelvic pain amongst those women with endometriosis was 3.81cm (SD 3.8cm), and for those women without endometriosis was 4.24cm (SD 3.82cm).

Discussion

Main Findings

This primary cohort study indicates that CA 125 ≥ 30 u/ml has a high accuracy for the detection of endometriosis in symptomatic women without evidence of other concurrent gynecological disease. CA 125 provides limited sensitivity for the detection

of endometriosis and a negative test cannot exclude endometriosis. In the absence of other accurate biomarkers, CA 125 ≥ 30 u/ml provides diagnostic confidence to both clinicians and patients.

Strengths

This study has robust design with adequate power. We prospectively recruited a small homogenous cohort of women with pain or subfertility and no known additional disease. We reduced recruitment bias by limiting recruitment to a single researcher and assay bias was minimized by the use of a single quality controlled NHS laboratory. We blinded a select group of surgeons working at a endometriosis specialist center to the outcome of the index test result. We limited interpretation bias by using a pre-defined validated cut-off for the analysis. We addressed clinical heterogeneity by excluding participants with other diseases known to cause a raised CA 125.

Limitations

This study has limitations. We sampled the index and reference standard at varied times during the menstrual cycle amongst women, including those on hormonal modulators. Although there is no clear influence of menstrual timing [17] or hormones [18] altering CA 125 levels this introduces clinical heterogeneity. CA 125 is known to be raised in other benign and malignant gynecological pathology. We attempted to control for this by excluding all those patients with prior USS or MRI evidence of leiomyoma, adenomyosis, and hydrosalpinges, benign non-endometriotic cysts. We excluded those

patients with a previous history of pelvic inflammatory or sexually transmitted disease. To minimize verification bias, the authors chose to restrict included patients to those with histologically confirmed endometriosis. This study was subsequently limited by the small number of included patients. There remain limitations with the reference standard (visualization and histological confirmation) used in this trial. The presence of occult microscopic endometriosis has been confirmed on visually normal peritoneum creating verification bias [19].

Comparison with existing literature

Previous primary studies and systematic reviews have demonstrated a limited role for the use of CA 125 in the detection of endometriosis. These studies suffered from significant verification bias (visual detection), design bias (case-control studies) and cohort heterogeneity (varied recruitment strategies) [1]. The sensitivity of CA 125 has repeatedly been demonstrated as poor with increasing accuracy associated with advancing stage of disease [1]. The search for an accurate non-invasive biomarker for endometriosis remains elusive [20,21] despite it being highlighted a research priority in 2009 [22].

Interpretation

As confirmed by Mol et al 1998, CA 125 is an important biomarker with a role as a rule-in test for women with pain or subfertility [23]. The sensitivity of this test remains poor, limiting its use to cohorts of symptomatic women with a high pre-test prevalence. The

diagnosis of women with pain or subfertility and a normal USS remains difficult and a CA 125 < 30 u/ml does not exclude endometriosis. Empirical use of the combined oral contraceptive pill remains an essential management strategy for women presenting with pain. This study demonstrates that when CA 125 ≥ 30 u/ml is used amongst a defined population with a narrow inclusion criteria for testing, a positive result provides a very high post-test probability. The high specificity minimizes false positive results and unnecessary treatment exposure from hormonal therapies or surgical procedures. The time from symptom onset to diagnosis and treatment remains a major concern for patients. The implementation of CA 125 in primary care or hospital settings as a point of care test for women with pain or subfertility and a normal USS may decrease delays in the diagnostic pathway, allowing women relief, liberation and legitimisation of their symptoms, together with access to support and an opportunity to discuss individualized medical or surgical management [1]. Further research is required amongst a population of women with pelvic pain or subfertility and a negative pelvic USS to assess its role in triaging treatment, access to specialist services, and reducing time to diagnosis or symptom control.

Conclusion

In the absence of a more accurate, non-invasive diagnostic test, CA 125 ≥ 30 u/ml can act as a rule-in test for the diagnosis of endometriosis amongst women presenting with symptoms of pain or subfertility.

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Conflicts of Interest

The authors report no conflicts of interest.

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Figure Legend

Figure 1. Flow of included participants

Study flow diagram.

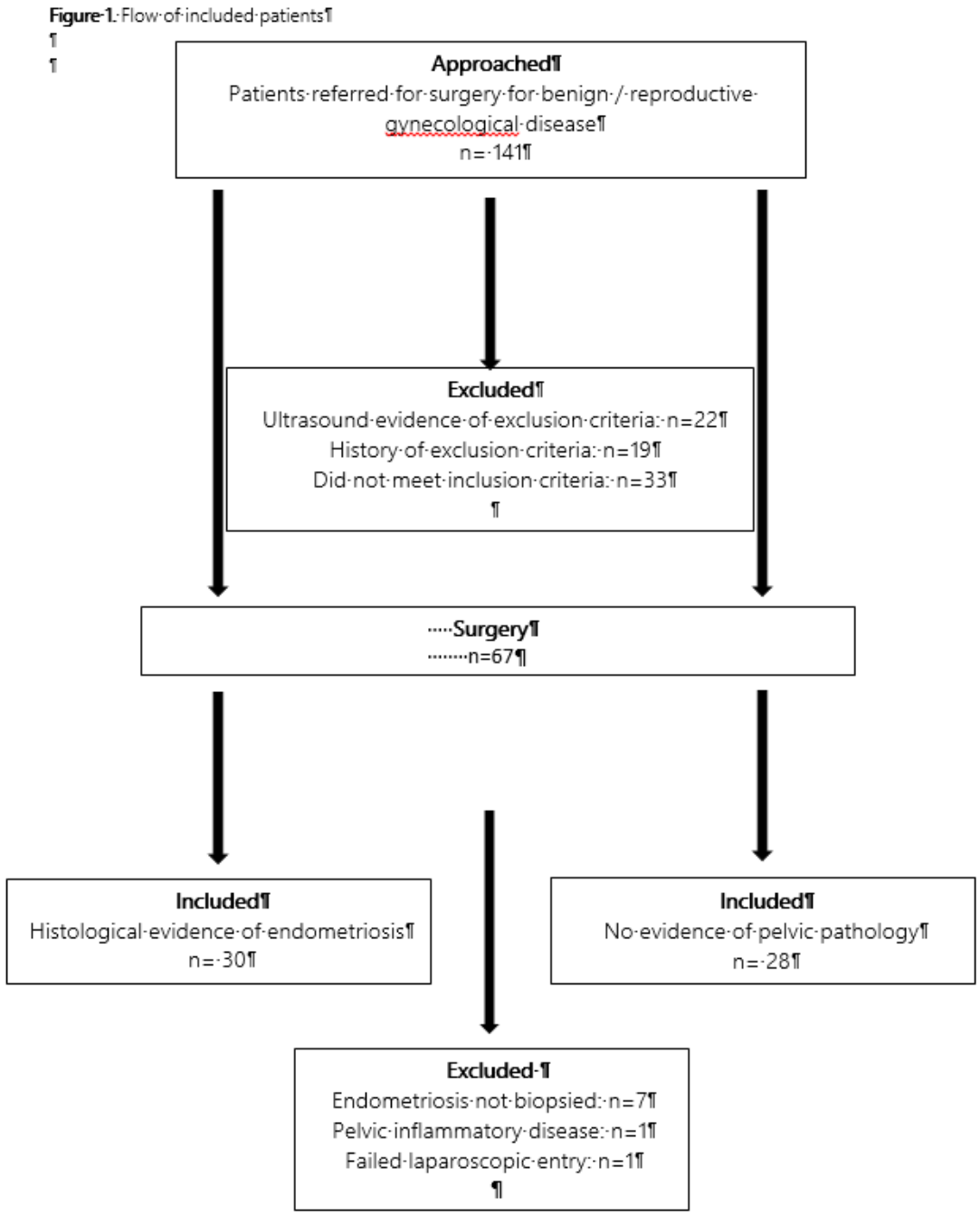
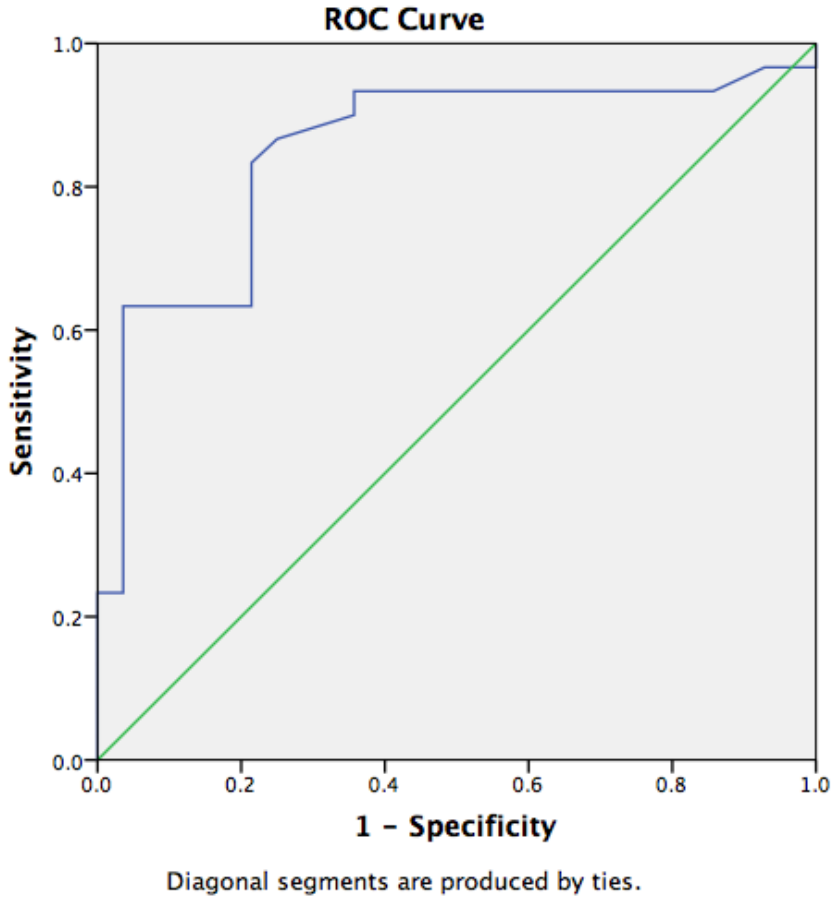


Figure 2. Receiver Operating Characteristics Curve

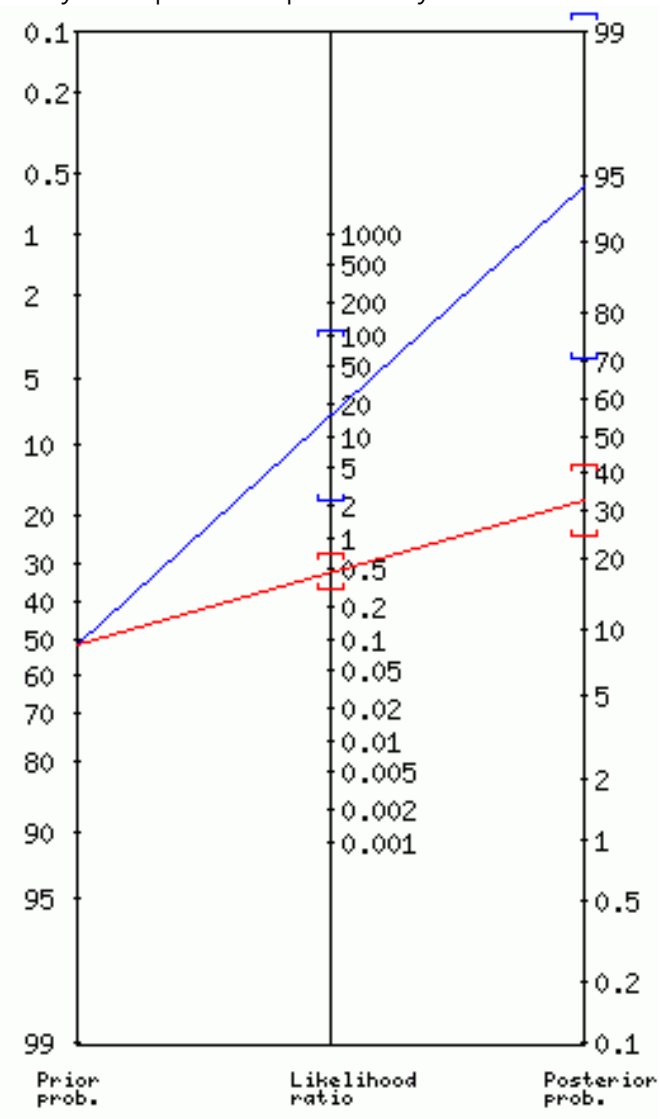
Sensitivity and specificity analysis of index test.



Area Under the Curve				
Test Result Variable(s): CA 125 u/ml				
			Asymptotic 95% Confidence Interval	
Area	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
.850	.054	.000	.744	.956
The test result variable(s): CA125 has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.				
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

Figure 3. Fagan's Nomogram

Analysis of post-test probability.



POSITIVE TEST:	
Positive Likelihood ratio:	15.75
95% confidence interval:	[2.26,112]
Post test probability (odds):	94% (17.1)
95% confidence interval:	[71%,99%]
NEGATIVE TEST:	

Negative Likelihood ratio:	0.45
95% confidence interval:	[0.30,0.68]
Post test probability (odds):	33% (0.5)
95% confidence interval:	[24%,42%]

Table 1. Participant Characteristics

Baseline Characteristics	Endometriosis (n=30)	Controls (n=28)
Mean Age, yrs	34.1	32.2
Primary Infertility, n (%)	14 (47%)	8 (29%)
Secondary Infertility, n (%)	3 (10%)	6 (21%)
Endometriosis Stage I-II, n (%)	17 (57%)	-
Endometriosis Stage III-IV, n (%)	12 (40%)	-
Mean CA 125 value u/ml	54.7 (SD 71.6)	16.2 (SD 7.97)
Hormonal contraceptive use, n (%)	5 (17%)	4 (14%)
Preoperative pain score		
Mean VAS (0-10cm) Dysmenorrhea	8.10 (SD 1.41)	6.49 (SD 2.97)
Mean VAS (0-10cm) Dyspareunia	5.26 (SD 3.31)	4.53 (SD 3.79)
Mean VAS (0-10cm) Dyschezia	3.77 (SD 3.41)	1.91 (SD 2.81)
Mean VAS (0-10cm) Chronic pelvic pain	3.81 (SD 3.80)	4.24 (SD 3.82)
Mean VAS (0-10cm) Dysuria	1.25 (SD 1.99)	0.73 (SD 1.72)