Diagnostic accuracy of Cancer Antigen 125 for endometriosis: A systematic review and meta-analysis

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Running title:
Diagnostic accuracy of CA 125 for endometriosis.

Abstract

Background: The development of a non-invasive and accurate diagnostic biomarker for endometriosis is urgently needed.

Objective: Evaluate the diagnostic accuracy of serum cancer antigen 125 (CA 125) for endometriosis.

Search Strategy: We searched: 1) EMBASE, 2) MEDLINE, and 3) Web of Science from inception to January 2016.

Selection Criteria: Diagnostic accuracy studies of serum CA 125 (index test) for histologically confirmed endometriosis (reference standard) were included.

Data Collection and Analysis

Two authors independently selected trials, extracted study characteristics and data. Methodological quality was assessed using Quality Assessment of Comparative Diagnostic Accuracy Studies (QUADAS-2) checklist.

Main Result(s): Twenty-two studies (16 cohort, six case-control), 3626 participants, were identified. Bivariate hierarchical models were used to pool accuracy data of 14 studies (2920 participants) using CA 125 ≥30 units/millilitre. Pooled specificity was 93% (95% CI 89% - 95%) and sensitivity 52% (95% CI 38% - 66%). CA 125 was significantly more sensitive for the diagnosis of moderate or severe endometriosis compared to minimal disease (63% 95% CI 47% – 77% vs. 24% 95%CI 19% - 32%, p value=0.001).

Conclusions: CA 125 performs well as a rule in test facilitating expedited diagnosis and ensuring investigation and treatment can be confidently tailored towards the management of endometriosis. Unfortunately, a negative test, CA 125 < 30 units / milliliter, is unable to rule out endometriosis.

Key words: endometriosis, non-invasive diagnosis, cancer antigen 125, biomarkers

Tweetable Abstract

Blood test CA 125: a rule-in test for the #diagnosis of women presenting with symptoms of #endometriosis

PROSPERO Registration Number: CRD42015017630.

Introduction:

Endometriosis, defined as the presence of endometrial glands and stroma located outside the uterus is characterized by pain and subfertility. Estimates of disease prevalence suggest endometriosis affects up to 75% of symptomatic women yet is commonly under-diagnosed. The gold standard diagnostic test is histological diagnosis. The invasive nature of diagnosis accounts for a significant delay in a formal diagnosis. This delay could result in disease progression, symptom deterioration and an annual societal cost of $49.6 Billion in the USA [1]. Evaluation of non-invasive diagnostic biomarkers has not identified an accurate test for the detection of endometriosis [2,3]. A rule in test could reduce time to diagnosis, provide psychological support, and provide reassurance to the clinician to offer tailored treatment options [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] and is the most investigated non-invasive diagnosis marker. Individual studies have methodological limitations in patient selection [7,9,13-21], poor conduct of the index test [7-12, 21-31], and poor conduct of the reference test [17-20, 32-57]. Two diagnostic review exists, however, the first is over fifteen years old including studies with high risk of verification bias[58] and the second is of poor methodological quality[59].

We conducted a meta-analysis to assess the diagnostic accuracy of CA 125 for histologically confirmed endometriosis.

Methods:

A protocol with explicitly defined objectives, criteria for study selection, approaches to assessing study quality, and statistical methods was developed and prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD42015017630, available online www.crd.york.ac.uk/prospero. We have reported the systematic review and meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [60].

A comprehensive and systematic literature review was undertaken searching: 1) EMBASE, 2) MEDLINE, and 3) Web of Science from inception to January 2016. We searched the register using MeSH and free text combinations with Boolean logic of the following search terms: endometrio*, test*, diagnos*, accura*, marker, screen*, detect*, CA 125, Cancer Antigen 125, CA-125, CA125. There were no language or date restrictions (Appendix S1).

Two reviewers (MH and JMND) independently screened titles and abstracts. They critically reviewed the full text of selective studies to assess eligibility. Any discrepancies between the reviewers were resolved by discussion. We included prospective and retrospective observational studies (cohort and case-control) assessing the diagnostic accuracy of pre-operative serum CA 125 to detect endometriosis confirmed by histology collected at robotic, laparoscopic, or open

surgery. We excluded studies that used visual confirmation of endometriosis as the reference standard, studies that only assessed ovarian cysts, and those where the comparator group included malignant disease.

Two reviewers (MH and JMND) extracted the data independently using a pilot-tested data extraction sheet. Information collected from each study included study design, setting, and participants. We extracted all relevant raw data from each study. Two reviewers (MH and JMND) independently assessed each study's methodological quality by using the Quality Assessment of Comparative Diagnostic Accuracy Studies (QUADAS-2) checklist — patient selection, conduct of the index test, conduct of the reference test, and patient flow. We considered studies to be of high quality if they sampled an appropriate patient spectrum, used consecutive recruitment, index test was performed before the reference standard, and all participants underwent the same reference standard [61]. The following were considered study qualities with potential to introduce bias; patients with a pre-operative ultrasound diagnosis of endometriosis, case-control studies, control groups that did not undergo the reference standard test, and studies with <85% histological confirmation of endometriosis. These were assessed with subgroup and sensitivity analysis.

Data was extracted for the number of true positives, true negatives, false positives, and false negatives for the index test at the documented threshold. Where data was unavailable the authors used the published sensitivities and specificities to calculate these data necessary to complete a 2 x 2 table. We actively contacted authors to seek clarification and requested missing data or additional data to complete our analysis [7,31,62,63]. Discrepancies between the reviewers (MH and JMND) were

resolved through discussion, by contacting the authors, or by consultation with a third reviewer (KSK).

Data synthesis was performed using a priori hypothesis described in the protocol. Where studies reported multiple cut off values for CA 125 we selected the closest value to the laboratory upper limit of normality (35 units / millilitre) for our analysis [64]. We explored variation in accuracy indices graphically using forests plots of sensitivity and specificity and ROC plane plots of sensitivity against specificity. As the studies used different cut-offs we grouped them in order to isolate subsets of studies using the same cut-off. In the case of no evidence of threshold effect within these subsets of studies, we fitted hierarchical bivariate random effects model [65] and obtained the following summary accuracy measures with corresponding 95% confidence intervals: sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio. Post-test probabilities were calculated based on pooled estimates of likelihood ratios and overall pretest odds based on published prevalence studies of endometriosis by clinical symptoms or signs. In case of evidence of threshold effects we summarized the analyses with the summary receiver operating characteristics curve. To investigate sources of heterogeneity, we performed subgroup analysis on the following pre-specified groups: 1) comparison of study design (cohort vs. case-control), 2) comparison of positive ultrasound findings for endometriosis (ovarian cyst vs. no cyst or no ultrasound) 3) comparison of revised American Fertility Score [66,67] (disease stage 1-2 vs. 3-4). Sensitivity analyses were performed to evaluate the impact on accuracy of excluding studies that had elements of verification bias, including 87% histological confirmation of endometriosis [11] and controls that did not undergo the reference standard [9]. We checked differences in sensitivity and specificity between subgroups by adding covariates to the bivariate model. Stata

168 software was used for statistical analyses. (StataCorp. 2013. Stata Statistical
169 Software: Release 13. College Station, TX: StataCorp LP).

170 Results:

171 Twenty-two studies included 3626 participants (Figure 1) [7-13,21-31,62,68-70]. Nineteen
172 prospective observational studies [7,8,10,12,13,22-31,62,68-70], and three retrospective
173 observational studies [9,11,21] were included for analysis. Two studies did not include
174 analyzable data and the authors did not respond to contact [7,62]. The studies were
175 relatively small (<300 participants), with the exception of Kitawaki 2005 [8], Cho 2008
176 [9], Yang [21] and Santulli 2015 [31] (Table 1). All studies were conducted in high-resource
177 settings [68]. Fifteen studies recruited patients from infertility clinics [7,10-13,22-
178 24,26,27,29,31,62,68,70] and eight studies included recruited patients from general
179 gynecology clinic or elective gynecological theatre sessions [8,10,12,21,23,26,29,31]. Twelve
180 studies reported including patients with pain symptoms [8,12,13,22-24,26,29-31,62,70]. Twelve
181 studies recruited patients with pre-operative imaging available indicating an ovarian
182 cyst [8-10,12,13,23,25,26,28,31,62,70]. Endometriosis was confirmed by histology collected at
183 either laparoscopic [10,11,13,21-24,27-31,62,67,70], laparoscopy or open [7,8,12,26,68] or did not
184 specify the route of surgery [9,25]. The staging of endometriosis was classified using
185 the revised American Fertility Society classification 1985 [66] or the revised American
186 Fertility Society classification 1997 [67]. Nine studies (954 participants) included
187 participants with minimal to mild endometriosis [7,9,10,12,13,26,27,62,68] and fourteen
188 studies (1479 participants) included participants with moderate to severe
189 endometriosis [7,9,10,12,13,21,24,26-28,30,62,68,69].

190 The authors judgment on risk of bias was used with the revised assessment tool:
191 Quality assessment of comparative diagnostic accuracy studies (QUADAS2) (figure

S1). Seventeen of the 22 studies had a low risk of bias owing to patient timing and flow. Two studies [9,21] were described as high risk of bias as the asymptomatic control group did not undergo surgery. All studies had a low risk of bias attributed to the reference standard, as this was deemed an objective histological assessment. One study was aware of the index test result prior to the reference standard [21], one study performed the index test following the reference standard [7], and a further study analyzed the index test after the reference standard [11]. These were deemed high risk of bias for conduct of the index test. Fourteen studies had a low risk of bias owing to patient selection; six were high risk owing to case-control design [7,9,13,21,23,25] and the remaining two were unclear [11,12]. Regarding applicability concerns, all studies were low risk for the index and reference standard. Twelve studies were low risk for patient selection and ten studies unclear owing to case-control design and inclusion of patients for tubal surgery, a group who may not routinely be screened for endometriosis [7-10,13,21,26,29,31,62].

Forest plots illustrate the variation in sensitivity and specificity between individual studies for the detection of pelvic endometriosis with serum CA 125 measurement (figure S2). Individual study sensitivities ranged from 0% [27] to 87% [30] and specificity from 51% [11] and 100% [70].

Fourteen studies, 2920 participants (1584 with endometriosis, 1336 controls) were meta-analyzed to assess the accuracy of CA 125 ≥ 30 unit / millilitre for the presence of endometriosis [8-10,12,13,21,24-27,30,39,69,70]. Serum CA 125 ≥ 30 unit / millilitrehad a pooled sensitivity of 52.4% (95% CI 37.9 - 66.4%) and specificity 92.7% (95% CI 89.4 - 95.1%) with no apparent correlation between sensitivity and specificity (Figure 2). A sensitivity analysis excluding an outlier study with 0% sensitivity [27] did not

significantly alter results (data not shown). When a mix of cut-off points for CA 125 were included into the analyses, a high variation in both sensitivity and specificity was observed with a clear threshold effect making accuracy estimates to this subgroup less useful (Figure 2).

Sources of heterogeneity were highlighted as study design (case-control versus cohort), the pre-operative ultrasound diagnosis of ovarian cysts and disease stage (Table S1). CA 125 showed higher sensitivity with increasing disease severity, 24.8% (95% CI 18.8 - 32.1%; stage I-II) versus 63.1% (95% CI 47.2 - 76.5%; stage III-IV). There were no significant differences in pooled sensitivity and specificity for the detection of endometriosis in the presence or absence of ovarian cysts or change in study design.

Sensitivity analyses excluding studies with verification limitations [9,11] did not change accuracy estimates of CA 125 for detecting the presence of endometriosis (Table 2).

Discussion:

Main Findings

CA 125 performs well as a rule in test, facilitating expedited diagnosis and ensuring investigation and treatment can be confidently tailored towards the management of endometriosis. Unfortunately, a negative test, CA 125 < 30 units / millilitre, is unable to rule out endometriosis.

Strengths and Limitations

This is the first prospectively registered review. We conducted a comprehensive search strategy, robust methodology, and statistical analysis. Previously published systematic reviews, are out of date [58], or associated with methodological bias arising from case-control studies[59] and verification bias arising from the reliance upon visual inspection which is now known to be inaccurate [72]. All studies included in this study reported the primary endpoint using the reference standard of histologically confirmed endometriosis.

Diagnostic reviews are not without limitations. There was wide variation observed in the sensitivity of CA 125 between individual studies. This is thought to be due to clinical heterogeneity, for example Mohammed et al and Yang et al evaluated a population of women with advanced endometriosis while Molo et al and Wild et al recruited purely form a fertility clinic setting. Several other gynecological diseases cause a rise in CA 125 including, ovarian epithelial carcinoma, leiomyoma, and pelvic inflammatory disease and often included studies did not adequately rule these conditions out. There was variation in CA 125 assay assessment which could introduce bias. We included case-control studies [7,9,13,21,23,25] which can have large discrepancies between the anticipated prevalence of the groups.

Interpretation

CA 125 performs well as a rule in test. This offers women, presenting for the first time with pain or infertility and a positive test, the confidence that an initial diagnosis is correct. This may decrease delays in the diagnostic pathway, allowing women relief, liberation and legitimization of their symptoms, together with access to support and an opportunity to discuss tailored medical or surgical management [4].

To minimize the false negative rate, the use of CA125 is limited to women with symptoms of endometriosis, where there is high suspicion of disease and a high prevalence of disease within the population. The indiscriminate use of CA 125 should be avoided in favor of a targeted rule-in test for symptomatic women and their clinicians wishing for further confidence in diagnosis, prior to delivering a therapeutic intervention. CA 125 performs poorly as a rule out test and 49% of those with endometriosis will have a negative test. This can cause uncertainty and confusion amongst all those with a negative test, potentially leading to unnecessary presumptive hormonal treatment. Alternative non-invasive biomarkers currently being investigated include human epididymis protein 4 [73], and miRNA [74] which provide potential for accurate biomarkers of the future. It is therefore unlikely that CA 125 will provide a lasting role as a non-invasive diagnostic tool for endometriosis. However, there is currently no validated, accurate test available with sensitivity > 75% and specificity >75% [75,76]. In the absence of a more accurate non-invasive test for the diagnosis of endometriosis, we recommend the use CA 125 > 30 u/ml as a rule-in test amongst symptomatic women with a negative ultrasound.

Conclusions:

In symptomatic women, the use of CA 125 $\geq$ 30 iU/ millilitre is highly specific for diagnosing endometriosis. This specific test can, when positive, provide earlier access to treatment options, reduce time to diagnosis, and anxiety amongst endometriosis sufferers. A CA 125 of less than 30 iU / millilitre does not exclude endometriosis and further investigation is required. We recommend further research

on alternative biomarkers that are both sensitive and specific for the diagnosis of endometriosis.

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Conflict of Interest:

The authors disclose no conflicts of interest. The ICMJE disclosure forms are available as online supporting information.

Author contributions:

MH, JMND, KSK & CJD developed the concept and design of the study.
MH & JMND undertook data acquisition.
MNP performed data analysis and interpretation.
All authors; MH, JMND, CJD, MNP, KSK were involved with drafting the article, final approval and agree to be responsible for its accuracy.

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Figure Legends

Figure 1. Flow of included studies.

Figure 2. Summary Receiver Operating Characteristic Curves (CA 125 ≥ 30 or <30 u/ml).

Table Legends

Table 1. Characteristics of Included studies

Table 2. Sensitivity analysis

643 **Supplementary Material Legends**

644

645 **Appendix S1.** Search Strategy.

646

647 **Table S1.** Sub-group analysis

648

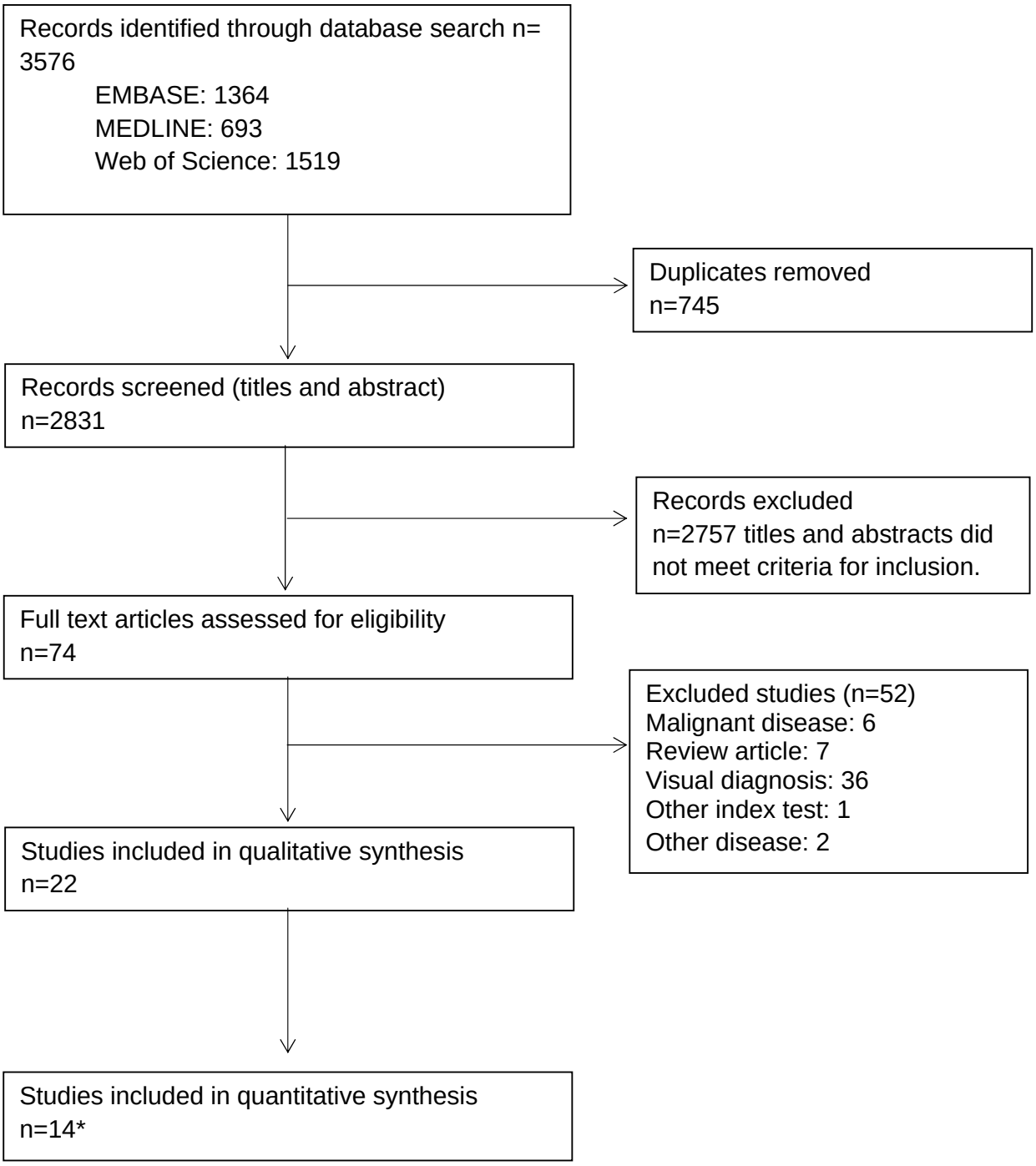
649 **Figure S1.** Quality Assessment using QUADAS2 Assessment tool.

650

651 **Figure S2.** Forest plots of sensitivity and specificity sorted in descending order of

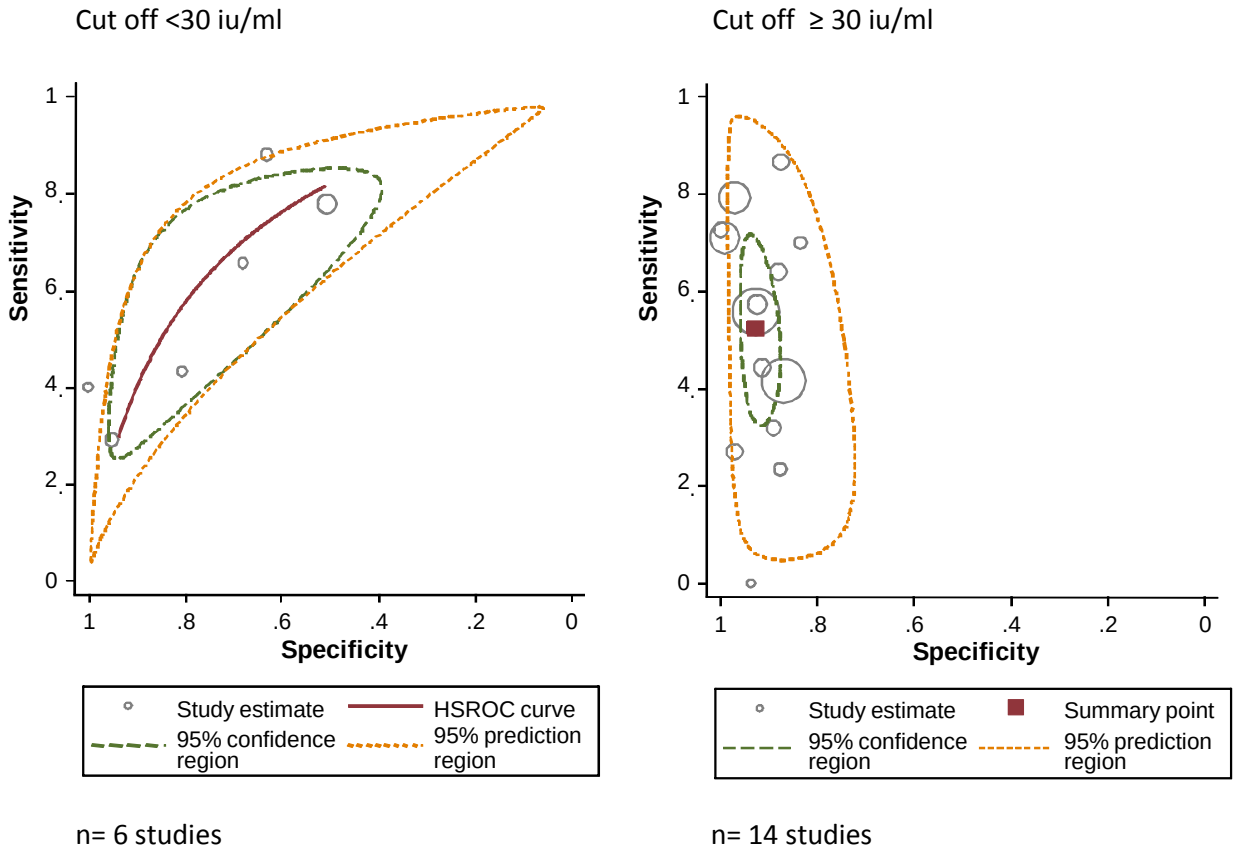
652 sensitivity, stratified by cut-off (CA 125 ≥ 30 or <30 u/ml).

Figure 1. Flow of included studies.



*8 studies with a CA 125 cut off value < 30 iu/ml were not meta-analyzed due to statistical variation with evidence of a threshold effect limiting accuracy estimates.

Figure 2. Summary Receiver Operating Characteristic Curves (CA125 <30 or ≥30 iu/ml)



n= 6 studies

n= 14 studies

Table 1. Characteristics of included studies.

Author	Year	Country	Participants	Study Design	Participant Characteristics	Ovarian Cysts included	Endometriosis staging criteria
Wild	1991	USA	93	cohort	infertility	no	rAFS 1985
Adamyan	1993	USSR	49	case-control	cysts	yes	rAFS 1985
Molo	1994	USA	35	cohort	Infertility	no	rAFS 1985
Abrao	1997	Brazil	50	case-control	Not specified / tubal reanastomosis	yes	rAFS 1985
Chen	1998	Taiwan	99	cohort	pain	no	rAFS 1985
Kitawaki	2005	Japan	350	cohort	gynecology referral / pain / cysts	yes	rAFS 1997
Amaral	2006	Brazil	52	cohort	infertility / pain / tubal ligation	no	rAFS 1997
Cho	2008	South Korea	760	case - control	elective gynecological surgery / cysts	yes	rAFS 1997
Gajbhiye	2008	India	77	cohort	infertility department / cysts	yes	rAFS 1997
Jing	2008	Japan	61	case - control	pain / Infertility / cysts	yes	rAFS 1997
Salahpour	2009	Iran	60	cohort	pain / infertility / miscarriage	no	rAFS 1997
Kurdoglu	2009	Turkey	127	cohort	pain / infertility / general gynecology / cysts	yes	rAFS 1997
Florio	2009	Italy	99	cohort	endometrioma vs other cysts	yes	rAFS 1997
Tokmak	2011	Turkey	88	cohort	cysts	yes	rAFS 1997
Vodolazkaia	2012	Belgium	296	cohort	infertility / biobank	no	rAFS 1997
Ramos	2012	Brazil	104	cohort	pain / infertility / tubal ligation / cysts	yes	rAFS 1997
Mohammed	2013	Egypt	60	cohort	pain / Infertility	no	rAFS 1997
Sayan	2013	Turkey	100	cohort	pain / infertility / general gynecology / tubal ligation / cysts	yes	rAFS 1997
Kubatova	2013	Turkey	73	cohort	pain / infertility / cysts	yes	rAFS 1997
Bilibio	2014	Brazil	97	case - control	pain / infertility / tubal ligation	no	rAFS 1997
Santulli	2015	France	685	cohort	pain / infertility / tubal surgery / cysts	yes	rAFS 1997
Yang	2015	China	309	case-control	elective gynaecological surgery	Yes	rAFS 1997

Table 2. Sensitivity analyses

		Studies	Endometriosis diagnosed/ controls	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR- (95% CI)	DOR (95% CI)
Cutoff Level ≥ 30	Total	14	1584/ 1336	52.4 (37.9; 66.4)	92.7 (89.4; 95.1)	7.2 (4.2; 12.3)	0.5 (0.4; 0.7)	14.0 (6.3; 31.4)
	Sensitivity analysis*	13	1353/ 807	51.8 (36.0; 67.3)	93.0 (89.0; 95.6)	7.4 (4.0; 13.5)	0.5 (0.4; 0.7)	14.2 (5.8; 34.7)

**without Cho 2008* [9]

		Studies	Endometriosis diagnosed/ controls	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR- (95% CI)	DOR (95% CI)
Cutoff Level < 30	Total	6	331/ 221	58.1 (39.7; 74.5)	79.4 (60.1; 90.8)	2.8 (1.6; 4.8)	0.5 (0.4; 0.7)	5.3 (3; 9.5)
	Sensitivity analysis*	5	214/ 140	54.6 (33.6; 74.2)	83.2 (67.8; 92.1)	3.3 (2.0; 5.4)	0.5 (0.4; 0.8)	6 (3.1; 11.3)

**without Vodolazkaia 2012* [11]