

CA 125 Levels Measured in Different Phases of the Menstrual Cycle in Screening for Endometriosis

ALTHEA O'SHAUGHNESSY, MD, JEROME H. CHECK, MD,
KOSROW NOWROOZI, MD, AND DEBORAH LURIE, PhD

Objective: To examine variations in CA 125 levels during the three phases of the menstrual cycle in women with and without endometriosis.

Methods: One hundred infertile women were studied prospectively. CA 125 levels were measured during menses and during the follicular and luteal phases before diagnostic laparoscopy. Subjects were divided into four groups: no evidence of endometriosis (35 women), stage I endometriosis (30 women), stage II endometriosis (21 women), and stages III and IV endometriosis (14 women).

Results: In the endometriosis groups, there was a significant difference in the mean CA 125 levels drawn at menses and those drawn in the follicular phase. In patients with severe endometriosis, there was also a difference in the mean CA 125 levels drawn at menses and in the luteal phase. This finding led to the development of a screening test based on the ratio of CA 125 levels at menses to levels in the follicular phase. The test based on this ratio (with a cutoff of 1.5) had a sensitivity of 62.5% and specificity of 75%, compared with a sensitivity of 26.8% and specificity of 100% for the test based on a single CA 125 level drawn at menses (with a cutoff of 35 U/mL).

Conclusions: CA 125 levels during menses are elevated compared with those during the follicular phase in patients with endometriosis. Screening tests based on the relationship of multiple CA 125 levels taken throughout the menstrual cycle were more sensitive for detection of endometriosis than tests based on a single CA 125 level. (*Obstet Gynecol* 1993;81:99-103)

CA 125 is a cell surface antigen expressed on certain cells derived from embryonic coelomic epithelium, the measurement of which aids in the diagnosis and clinical follow-up of patients with ovarian carcinoma.¹

From the Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Cooper Hospital/University Medical Center, Robert Wood Johnson Medical School at Camden, The University of Medicine and Dentistry of New Jersey, Camden, New Jersey.

Elevation of CA 125 levels has also been noted in patients with other benign conditions of the pelvis such as endometriosis, myoma, adenomyosis, acute pelvic inflammatory disease, and ovarian cysts.²⁻⁸ Levels are also above normal in pregnant women,⁹ and marked elevations of CA 125 in the early first trimester have sometimes been associated with poor fetal outcome.¹⁰

Routine measurement of CA 125 in women with infertility or pelvic pain remains controversial because of its low level of sensitivity, particularly in patients with mild endometriosis. Patients with severe endometriosis more often have elevated CA 125 levels.² Case reports and some studies have found a dramatic fall in CA 125 levels after surgical or medical therapy, allowing its use in clinical follow-up.^{3,11} Another report did not confirm these findings, demonstrating instead poor clinical correlation with the fall in CA 125 levels after treatment.¹²

This controversy may stem from several sources. CA 125 values may vary when measured at different phases of the menstrual cycle. Some studies have reported elevated levels during menses.¹³ Furthermore, researchers may use different cutoff values. For example, most authors consider a level above 35 IU/mL abnormal, but some have used a cutoff of 16 IU/mL to increase sensitivity.¹⁴ Lower values may improve sensitivity but will compromise specificity.

This prospective study attempted to identify variations in CA 125 levels during the different phases of the menstrual cycle in both normal and surgically confirmed endometriosis subjects.

Materials and Methods

One hundred consecutive infertile women were studied prospectively. They were divided into four groups

based on the results of diagnostic laparoscopy: 35 had no evidence of endometriosis, 30 had stage I endometriosis, 21 had stage II endometriosis, and 14 had severe endometriosis (stages III and IV). No subject declined participation in this study. We used the revised American Fertility Society classification of endometriosis for staging.¹⁵

Before laparoscopy, CA 125 was measured two or three times. Laparoscopy was performed by two surgeons who were blinded to the CA 125 values. The procedure was carried out as part of the infertility workup or in the presence of clinical symptoms that could be related to endometriosis. The diagnosis of endometriosis at laparoscopy was not confirmed by biopsy. Serum CA 125 levels were measured during the menstrual, follicular, and luteal phases of the menstrual cycle. The subjects were not taking any medication when CA 125 levels were measured. The phase of the cycle was determined by ultrasound scan and estradiol (E2) and progesterone levels; the luteal phase was diagnosed when progesterone levels were above 5 ng/mL.

The CA 125 assay used was a simultaneous sandwich, solid-phase radioimmunoassay system (Centocor, Inc., Malvern, PA) as reported previously.¹⁰ Samples were run in duplicate and the average response calculated. If the duplicates varied by more than 10%, the sample was re-assayed. Because all the samples from the same patient were not run in the same assay, we assessed inter-assay variability, which was 11.83%.

Two-way analysis of variance with repeated measures was used to compare the mean CA 125 levels by group and phase of the cycle. Significant differences between groups and phases were further analyzed using the Tukey method of post-hoc tests. All tests were done at the .05 level of significance.

Screening tests for the detection of endometriosis based on the relationship of multiple CA 125 levels in the cycle were proposed and evaluated in terms of their sensitivity, specificity, predictive values, and efficiency. They were compared to the performance of the screening test based on a single menses CA 125 level with a cutoff of 35 U/mL.

Results

The mean (\pm standard deviation) ages for each group were 34 ± 8.5 years for no endometriosis, 35.4 ± 3.9 years for stage I, 35.4 ± 3.4 years for stage II, and 36 ± 4.0 years for severe endometriosis; no significant difference was noted. Other pelvic disease found at surgery included leiomyoma. The numbers of women with myomas did not differ significantly among the groups: 8.6% (three of 35) for no endometriosis, 6.7%

Table 1. Comparison of Mean Serum CA 125 Levels by Stage of Endometriosis and Phase of Menstrual Cycle*

Stage of endometriosis	Phase of menstrual cycle		
	Menses	Follicular	Luteal
None (N = 18)	15.8 \pm 7.3 [†]	12.2 \pm 4.1 [†]	13.2 \pm 4.2
I—Mild (N = 11)	22.6 \pm 12.4 ^{††}	13.6 \pm 4.2 [§]	15.4 \pm 5.4
II—Moderate (N = 13)	33.8 \pm 38.2 ^{††}	19.9 \pm 18.6 ^{†§}	23.9 \pm 27.7
III or IV—Severe (N = 7)	68.7 \pm 43.0 ^{†††}	31.1 \pm 15.2 [§]	26.3 \pm 15.7 [§]

* Tukey post-hoc tests were performed to compare the mean CA 125 levels between endometriosis stages at each phase of the cycle, and between the phases of the cycle within each stage of endometriosis.

[†] At this phase, $P < .05$ vs the severe group.

^{††} For this group, $P < .05$ vs the follicular phase.

[§] For this group, $P < .05$ vs the menses phase.

^{†††} For this group, $P < .05$ vs the luteal phase.

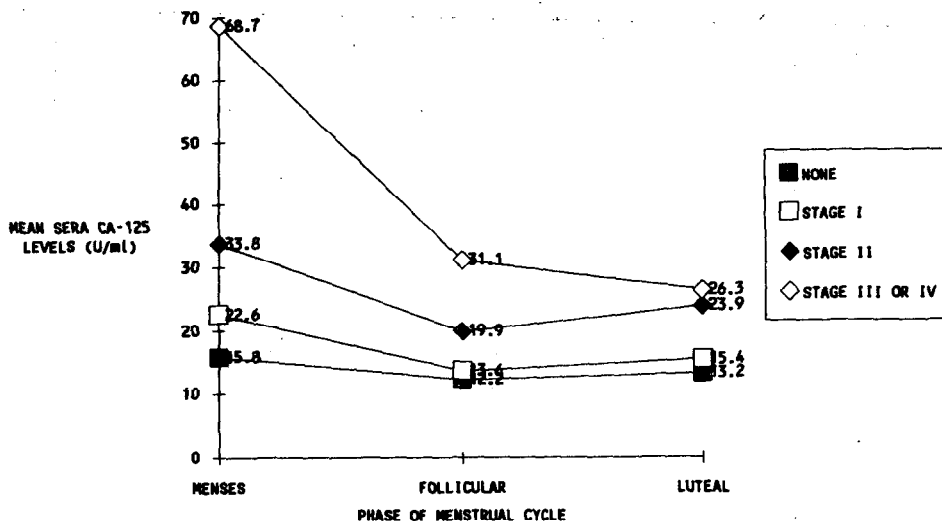
(two of 30) for stage I, 19% (four of 21) for stage II, and 14% (two of 14) for stages III and IV. Only two patients with myomas, both with stage II disease, had elevated CA 125 levels.

CA 125 levels were drawn in all three phases of the cycle for 49 of the 100 patients. Missing data occurred at the same rate in all groups: 51.4% in the group with no endometriosis, 36.7% in stage I, 44.8% in stage II, and 50% in severe endometriosis. The analysis of variance with repeated measures, performed using those subjects with complete data, showed that there was a significant interaction between group and phase of the cycle (Table 1). This indicates that the levels of CA 125 found throughout the cycle are not the same for each stage of endometriosis. Further analysis using Tukey post-hoc tests revealed that women with no endometriosis had the same CA 125 levels throughout the cycle. In the stage I and II groups, there was a significant difference between the CA 125 levels at menses and those in the follicular phase, but no difference between menses and luteal levels or follicular and luteal levels. In the severe-endometriosis group, menses levels were higher than follicular levels and luteal levels, but follicular and luteal levels were the same. Figure 1 illustrates the pattern of mean CA 125 levels throughout the cycle for each group.

All available data were used to evaluate screening tests designed to detect endometriosis. The screening test based on the menstrual CA 125 level (with a cutoff of 35 U/mL) had high specificity (100%) but very low sensitivity (26.8%) when used to detect patients with any stage of endometriosis. When used to predict a certain stage of endometriosis, its sensitivity was 13.3% for stage I, 37.5% for stage II, and 66.7% for stages III and IV.

In an attempt to develop a test that had higher

Figure 1. Comparison of the mean serum CA 125 levels by phase of the menstrual cycle and stage of endometriosis in subjects sampled at each of the three intervals.



sensitivity, we considered tests based on the relationship of multiple CA 125 levels. Because the luteal phase levels did not differ by group, we concentrated on tests relating the menses and follicular phase levels. The tests proposed for evaluation were based on the ratio of menses to follicular CA 125 levels. In this test, a ratio greater than or equal to the cutoff value was defined as a positive result (ie, endometriosis), and a ratio below the cutoff was considered a negative result (ie, no disease). The goal was to determine the cutoff that would increase sensitivity without decreasing the specificity below 75%.

The sensitivity and specificity of the screening test based on the ratio of menstrual to follicular CA 125 levels were evaluated for tests using a cutoff value beginning with 1.0 and increasing to 2.0 in increments of 0.25. As the cutoff increased from 1.0 to 2.0, the test sensitivity decreased from 83.8 to 29.7%, while the specificity increased from 40 to 95%. The cutoff value yielding the highest sensitivity with a specificity over 75% was 1.5. Table 2 presents the test sensitivity and specificity for each cutoff value.

The test based on a cutoff of 1.5 was further evaluated to determine its efficiency in identifying women with early stages of endometriosis. Table 3 presents the sensitivity, specificity, predictive values, and effi-

ciency of this test for each stage of endometriosis. At each stage of endometriosis, this test had higher sensitivity than the test based on a single CA 125 level.

Discussion

The controversy surrounding the use of CA 125 levels in screening for endometriosis stems from the wide variability in the sensitivity and specificity values quoted by numerous authors. Our overall sensitivity when using a single menstrual CA 125 level was only 26.8%. Lanzone et al¹² noted an overall sensitivity of 53% when using one random CA 125 value, but their study population consisted mainly of patients with moderate and severe disease, which tends to increase the level of sensitivity. Our study had a greater number of subjects with mild and moderate endometriosis. The use of different cutoff values will also account for differences in sensitivity levels. Pittaway¹¹ also quoted a sensitivity value of 53% using a threshold of 16 IU/mL. As pointed out by Pittaway,¹⁶ using 16 IU/mL as the upper limit of normal requires certain modifications of the standard curve. The standard curve used in our study did not allow speculation regarding changes in specificity or sensitivity had 16 IU/mL been used as a cutoff value.

A majority of studies measuring CA 125 values in patients with endometriosis have failed to specify when levels were drawn during the menstrual cycle. Other investigators have described elevation of serum CA 125 levels during menses. Pittaway and Jamil¹³ first demonstrated this in a retrospective study. Only one of nine in the non-endometriosis population had a level higher than 65 U/mL during menses, and the remainder had values below 35 U/mL. The one patient

Table 2. Sensitivity and Specificity for a Test Based on the Ratio of CA 125 Levels for Various Cutoffs

Cutoff	Sensitivity (%)	Specificity (%)
1.00	83.8	40.0
1.25	73.0	65.0
1.50	62.5	75.0
1.75	51.3	85.0
2.00	29.7	95.0

Table 3. Sensitivity, Specificity, Predictive Values, and Efficiency of Screening Tests for Endometriosis Based on the Ratio of Serum CA 125 Levels

Type of endometriosis	Test criteria*	Sensitivity (%)	Specificity (%)	Predictive value		Efficiency of test (%)
				Positive (%)	Negative (%)	
Stage I	A	13.3	100.0	100.0	56.4	64.9
	B	50.0	75.0	58.3	68.2	64.7
Stage II	A	17.6	100.0	100.0	61.1	69.4
	B	62.5	75.0	66.7	71.4	69.4
Stage III or IV	A	66.7	100.0	100.0	88.0	90.0
	B	85.7	75.0	54.5	93.8	77.8

* Criteria for a positive test were A: menstrual sera CA 125 levels >35 U/mL; B: ratio of follicular to menstrual sera CA 125 levels \geq 1.5.

who had high levels both before and during menses could have had other pelvic disease to account for such elevated values. In our study, none of the non-endometriosis patients had levels exceeding 35 U/mL. Therefore, measuring levels during menses did not compromise specificity and may, in fact, have increased sensitivity.

Other factors that may elevate CA 125 levels can also account for variations in specificity and sensitivity. In our study, leiomyoma was the only pelvic disease besides endometriosis that could account for elevations in CA 125 values. These findings did not seem to have a significant impact on the results or conclusions. Unlike others,⁵ we did not find an association between myomas and abnormal CA 125 levels, but this may be due to the small numbers involved.

Measurement of CA 125 levels during the different phases of the menstrual cycle has been studied in "normal" populations, in which values have been shown to remain stable or to increase with follicular development.¹⁷⁻¹⁹ Variation in CA 125 levels during the three phases of the menstrual cycle in a population with endometriosis has not been described. We found significant differences in CA 125 levels when comparing those drawn at the time of menses with those obtained during the follicular or luteal phases only in the endometriosis population. Reasons for these unique fluctuations remain unclear. Pittaway⁶ suggested that elevated CA 125 levels in women with endometriosis may stem simply from an increase in tissue containing CA 125. Elevations specifically during menses may result from retrograde flow and increased peritoneal inflammation, which may be more pronounced in patients with endometriosis than in women without disease.

The results of our study demonstrate that the exclusive use of the menstrual CA 125 level or the ratio of menstrual to follicular phase levels is not a reliable method of screening for endometriosis. Surgical confirmation for diagnosis and staging cannot be elimi-

nated. Measurement of CA 125 levels during both menses and the follicular phase seemed to enhance sensitivity in detecting patients with endometriosis, particularly those with stages I and II, compared with the use of a single CA 125 level. For this reason, we propose that these tests may allow earlier recognition of endometriosis, particularly in infertile and asymptomatic patients. In addition, these tests may prove helpful for detecting recurrence of endometriosis in post-therapy follow-up, thus allowing more timely intervention. A prospective study of CA 125 levels during menses and the follicular phase before and after surgical therapy is under way. A long-term prospective study correlating these various blood levels and pregnancy rates in an infertile population would prove most helpful in determining the ultimate value of measuring CA 125 levels in the menstrual and follicular phases in this population.

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Address reprint requests to:
Althea O'Shaughnessy, MD
 8002 East Greentree Commons
 Marlton, NJ 08053

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