

Use of a new CA 125 assay in the diagnosis of endometriosis*

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Serum CA 125 concentrations are elevated in some women with endometriosis; however, the low sensitivity and specificity of the assay have made it impractical to use it as a diagnostic test for endometriosis. Recently a second-generation CA 125 assay has been developed. The purpose of this study was to compare serum CA 125 concentrations in women with and without endometriosis using both the older assay and the new CA 125 assay and to determine if the new assay concentration improves the clinical utility of CA 125 in the diagnosis of endometriosis. A total of 123 patients with endometriosis, pelvic pain or infertility were enrolled. Blood for CA 125 was drawn in the cycle preceding laparoscopy or laparotomy, and concentrations found by the older and newer assays were correlated with the patients' endometriosis stage using the revised American Fertility Society classification of endometriosis. The CA 125 concentrations determined by the new assay were highly correlated with concentrations determined by the older assay in patients with and without endometriosis ($r = 0.96$). The sensitivity and specificity were slightly improved using the new CA 125 assay; however, this assay did not dramatically improve detection of endometriosis.

Key words: CA 125/new CA 125 assay/endometriosis

Introduction

CA 125 is the antigenic determinant, defined by the OC 125 monoclonal antibody, obtained by somatic hybridization of B lymphocytes from mice immunized with cells from an ovarian carcinoma cell line. CA 125 is based on the detection of a high molecular weight glycoprotein that is expressed on derivatives of the coelomic epithelium (Bast *et al.*, 1981; Jacobs and Bast, 1989). The hypothesis that endometriosis implants are derived from coelomic epithelium led numerous investigators to study serum CA 125 concentrations in patients

with endometriosis. Although CA 125 values in patients with advanced endometriosis are elevated, the sensitivity of a single CA 125 concentration as a diagnostic test for minimal and mild disease has been disappointing, ranging from 0.16 to 0.52 (number of patients with positive test divided by number of patients with all stages of endometriosis) (Barbieri *et al.*, 1986; Hornstein *et al.*, 1992; O'Shaughnessey *et al.*, 1993). Some improvement in sensitivity and specificity was noted, however, in one study by using a ratio of menstrual and follicular phase CA 125 concentrations (O'Shaughnessey *et al.*, 1993). The assay is less precise for CA 125 concentrations <20 U/ml due to a non-linear standard curve. A proposed modification of the curve has been described (Pittaway, 1989).

Recently, a second-generation CA 125 assay has been developed. This assay utilizes a new CA 125 capture antibody, termed M 11, absorbed on polystyrene beads to bind molecules containing OC 125 reactive determinants. M 11 is a murine monoclonal antibody which immunoprecipitates multiple sub-species of CA 125 (Hardardottir *et al.*, 1990; O'Brien *et al.*, 1991). It recognizes a different epitope on the high molecular weight glycoprotein which expresses OC 125 (Kenemans *et al.*, 1993). Utilizing this new antibody, the new assay measures CA 125 serum concentrations with greater precision, particularly at CA 125 concentrations <35 U/ml. Improved precision of the new assay has been noted in both apparently healthy women and in women with gynaecological malignancies (Bonfer *et al.*, 1994). Since patients with endometriosis tend to have normal to slightly elevated concentrations of CA 125 using the original assay, it was hoped that the new assay would improve the clinical utility of CA 125 for the diagnosis of endometriosis. Given the poor sensitivity of the existing CA 125 assay in the detection of endometriosis, this study was undertaken with two purposes in mind: (i) to compare the serum CA 125 concentrations determined by both assays in women with and without endometriosis, and (ii) to determine if the new assay improves the clinical utility of CA 125 in the diagnosis of endometriosis.

Materials and methods

A total of 123 patients with the pre-operative diagnosis of endometriosis, pelvic pain, or infertility were enrolled in the study. Of these, 66 were recruited from the Fertility and Endocrine Unit of the Brigham and Women's Hospital, Boston, MA, USA, and 57 came from the Pennsylvania private practice of the Reproductive Endocrine/Infertility Service at the Cooper Hospital University Medical Center, Camden, NJ, USA. Patients had blood drawn in the early follicular phase of

*Presented in part at the Fourth World Congress on Endometriosis, Salvador, Brazil, May 25-28, 1994.

the cycle preceding laparoscopy or laparotomy, as part of investigations on the menstrual cyclicity of CA 125 in endometriosis (Hornstein *et al.*, 1992; O'Shaughnessy *et al.*, 1993). The operating surgeon was not sure of the patients' CA 125 concentration at the time of surgery. These studies were approved by the appropriate hospital Institutional Review Boards. The blood samples were immediately centrifuged and the serum stored at -70°C for later analysis. At surgery the diagnosis was recorded, and patients found to have endometriosis were classified according to The American Fertility Society revised classification of endometriosis (American Fertility Society, 1985).

Of the 123 patients enrolled, 49 were found to have no endometriosis at surgery. Of the 74 patients with endometriosis, 33 had stage I, 21 had stage II, and 20 had stage III or IV disease. For both the older and new assays, CA 125 concentrations were determined by immunoradiometric assay (Centocor, Malvern, PA, USA), and were expressed in arbitrary units based on a primary reference standard. The interassay and intra-assay coefficients of variation (CVs) were 8.3 and 12.1% for the older CA 125 assay and 5.2 and 7.5% for the new CA 125 assay respectively. Matched concentrations from the two assays were compared using the Pearson correlation coefficient and the paired *t*-test was used to determine whether the mean difference between the older and newer CA 125 assays was significantly different from zero.

Sensitivity and specificity for endometriosis were calculated using values from both types of assay. The positive and

negative predictive values of the CA 125 assays used in this study as a screening tool for endometriosis were dependent upon the prevalence of endometriosis in women with infertility and pelvic pain (our source population for this study). We and other investigators have observed about a 40% prevalence of endometriosis in women presenting with infertility and pelvic pain, and of these, about one-third are likely to be diagnosed with stage III or IV disease (Olive and Schwartz, 1993). Similar prevalence rates of endometriosis have been observed in adolescent girls (Vercellini *et al.*, 1989). With the use of these prevalence estimates and our calculated sensitivity and specificity, we estimated the predictive value of the two CA 125 assays (Weiss, 1986).

Results

The new CA 125 assay concentrations were highly correlated with concentrations determined by the older assay for all subjects ($r = 0.96$). This high degree of correlation was true for patients without endometriosis ($r = 0.93$) and for each of the stages of endometriosis studied (Table I). Overall, both in patients without endometriosis, and in patients with each of the stages of endometriosis evaluated, the concentrations determined by the new assay were consistently higher than those determined by the older assay (Table I). However, none of the differences were statistically significant.

The clinical utility of the two assays in the diagnosis of endometriosis was studied by comparing the sensitivity and specificity of each CA 125 assay. A value of 35 IU/ml was chosen as the upper limit of normal. The use of the cut-off value of 35 U/ml was determined by construction of receiver-operator characteristic (ROC) curves for both assays. As no dramatic improvement was noted in the trade-off between sensitivity and specificity for either test over the range of values studied, the historically used value of 35 U/ml was chosen for this study. Sensitivity was defined as the number of patients with all stages of endometriosis who had concentrations >35 U/ml (determined by either the old or new assay), divided by the total number of patients with endometriosis (Table II). Specificity was defined as the number of patients who did not have endometriosis and had CA 125 concentrations <35 U/ml divided by the total number of patients without endometriosis. Sensitivity and specificity were also calculated in this manner for patients with stages III and IV endometriosis,

Table I. CA 125 concentrations by stage of endometriosis using older and new assays. Values are mean \pm SD

Group	Older CA 125 assay (U/ml)	New CA 125 assay (U/ml)	r^a
All subjects ($n = 123$)	21.1 \pm 23.3	24.9 \pm 24.4	0.96
No endometriosis ($n = 49$)	16.2 \pm 14.0	18.9 \pm 11.5	0.93
Endometriosis stage I ($n = 33$)	14.1 \pm 9.4	17.9 \pm 9.0	0.97
Endometriosis stage II ($n = 21$)	23.0 \pm 26.5	26.0 \pm 23.5	0.96
Endometriosis stage III/IV ($n = 20$)	42.8 \pm 38.2	49.8 \pm 44.0	0.97

^aPearson correlation coefficient.

Table II. Reliability of both older and new CA 125 assay for the diagnosis of endometriosis^a

Screening Measure	Older CA 125 assay		New CA 125 assay	
	all endometriosis	stages III/IV	all endometriosis	stages III/IV
Sensitivity	0.16 (0.08, 0.24)	0.40 (0.19, 0.61)	0.23 (0.11, 0.35)	0.60 (0.39, 0.81)
Specificity	0.92 (0.84, 0.99)	0.92 (0.84, 0.99)	0.94 (0.87, 0.99)	0.94 (0.87, 0.99)
PV ⁺	0.57 (0.33, 0.81)	0.43 (0.15, 0.71)	0.72 (0.52, 0.92)	0.60 (0.35, 0.85)
PV ⁻	0.62 (0.53, 0.71)	0.91 (0.84, 0.98)	0.65 (0.56, 0.74)	0.94 (0.88, 0.99)

^aPositive predictive value and negative predictive value were estimated from the sensitivity, specificity and an estimated 40% prevalence of endometriosis among women presenting to infertility clinics with pelvic pain, and a 13% prevalence of stage III or IV endometriosis among the same population; 95% confidence intervals are given in parentheses.

omitting patients with lower stage endometriosis from the sensitivity and specificity analyses.

Based on our past clinical assessment of this population, ~40% of women were diagnosed with endometriosis. Therefore, we estimated the positive and negative predictive values of the CA 125 assays with the use of this prevalence estimate, and our calculated sensitivity and specificity data (Weiss, 1986). For all patients with endometriosis, there was a slight, but not statistically significant improvement in sensitivity and specificity and positive and negative predictive values using the new assay compared with the older CA 125 assay. In addition, small improvements were seen in all these parameters using the new assay for stage III and IV disease (Table II). These differences also failed to reach statistical significance.

Discussion

Although CA 125 concentrations are elevated in women with advanced endometriosis, the low sensitivity and specificity of the current CA 125 assay have limited its applicability in the diagnosis of endometriosis. The new CA 125 assay uses a new capture antibody yielding greater precision in the lower ranges of the assay curve. The better reproducibility of the new assay suggested that this assay might improve the clinical utility of serum CA 125 measurements in the diagnosis of endometriosis.

The major findings of this study indicate that while new CA 125 assay concentrations are closely correlated to those of the older CA 125 assay, the improvement in the ability to differentiate between patients with and without endometriosis is small. The sensitivities and specificities reported in this study agree with our previous findings and are comparable to those reported by other investigators using the older CA 125 assay (Schenken *et al.*, 1990).

For all stages of endometriosis, reported sensitivities range from 0.06 to 0.14, with specificities of 0.91 to 0.97, and for stage III–IV disease from 0.20 to 0.54 and 0.91 to 0.97 for sensitivity and specificity respectively, using a cut-off value of 35 U/ml (Barbieri *et al.*, 1986; Patton *et al.*, 1986; Koninckx *et al.*, 1992). Improved sensitivity but reduced specificity can be obtained using a lower normal limit of 16 U/ml (Pittaway and Fayez 1986; Pittaway and Douglas, 1989).

This study used a CA 125 value of 35 U/ml as the upper limit of normal based on ROC analysis of the data for both assays, and historical precedent. The recent evolution of a new one-step enzyme immunoassay for CA 125 using the OC 125 antibody found 1% of normal women to have a CA 125 concentration >48 U/ml (Kenemans *et al.*, 1992). The proliferation of new test kits for CA 125 makes an understanding of assay characteristics and clinical cut-off values vitally important for the proper interpretation of the test.

Acknowledgements

This work was supported in part by a grant from Centocor, Inc., Malvern, PA, USA. Thanks go to Dr Paul Kaplan of Centocor, who kindly performed the assays.

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Received on September 28, 1994; accepted on January 20, 1995