Usefulness and limits of CA-125 in diagnosis of endometriosis without associated ovarian endometriomas

Jo Kitawaki1, Hiroaki Ishihara, Hisato Koshiba, Miyo Kiyomizu, Mariko Teramoto, Yui Kitaoka and Hideo Honjo

Department of Obstetrics and Gynecology, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto 602-8566, Japan

1To whom correspondence should be addressed at: Jo Kitawaki, M.D., Ph.D., Department of Obstetrics and Gynecology, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kamigyō-ku, Kyoto 602-8566, Japan
E-mail: kitawaki@koto.kpu-m.ac.jp

BACKGROUND: The aim of this study was to evaluate the diagnostic significance of CA-125 for endometriosis without ovarian endometriomas. METHODS: Preoperative serum CA-125 levels were measured in 775 consecutive women diagnosed by laparoscopy or laparotomy with endometriosis, adenomyosis, leiomyomas, or normal pelvis.
RESULTS: Receiver operating characteristic curve analysis revealed that the area under the curve for endometriosis without endometriomas was 0.788, significantly smaller than that for endometriosis with endometriomas (0.935, P < 0.05). In diagnosis of endometriosis without endometriomas, both the maximal accuracy of 78.8% and the maximal diagnostic value of 61.2% were obtained at the cutoff value of 20 U/mL. Negative predictive value was 78.0% at the cutoff value of 20 U/mL, whereas positive predictive value was 92.9% at the cutoff value of 30 U/mL. This range is clearly superior to the empirical single cutoff of 35 U/mL. CONCLUSIONS: In the diagnosis of endometriosis without endometriomas, combined use of two cutoff values for CA-125, 20 and 30 U/mL, provides improved diagnostic performance. However, the accuracy of using only CA-125 testing for diagnosis is still limited. Serum CA-125 testing can be done during initial screenings of women with possible endometriosis.

Key words: adenomyosis/CA-125/diagnosis/endometriosis/leiomyoma/ovarian endometriomas

Introduction

One of the difficulties in the diagnosis of endometriosis is that macroscopic and histologic demonstration of lesions in the abdominal cavity by laparotomy or laparoscopy is essential. Before establishment of diagnosis with surgery, a less invasive test is therefore important for initial screening of possible cases of endometriosis. Recent technical progress in transvaginal ultrasonography (Guerriero et al., 1998; Patel et al., 1999; Dessole et al., 2003) and magnetic resonance imaging (Takahashi et al., 1994; Bis et al., 1997; Kinkel et al., 1999; Stratton et al., 2003) improves diagnostic accuracy. These tools, together with symptoms and pelvic examination, appear to detect ovarian endometriomas with high sensitivity and specificity; however, current techniques are still limited in detecting small peritoneal lesions (Eskenazi et al., 2001). In addition to noninvasive imaging technologies, several biological markers for endometriosis can also be applicable to clinics (Kitawaki et al., 1999; Bedaiwy et al., 2002; Gagne et al., 2003). In this context, a less invasive test is needed that can be used as the initial screening for cases of endometriosis without ovarian endometriomas.

Serologic testing for CA-125 has been widely used for detection of endometriosis and monitoring of progressive disease (Barbieri et al., 1986; Pittaway and Fayez, 1986; Fedele et al., 1989; Koninckx et al., 1996; Medl et al., 1997; Cheng et al., 2002). Although the standard cutoff value of 35 U/mL was initially set to detect epithelial ovarian cancer (Bast et al., 1983), a decisive cutoff value for screening of endometriosis has not been set because serum level does not necessarily correlate with severity of disease. Indeed, serum levels in women with mild endometriosis are often lower than those in women without endometriosis. A meta-analysis based on 23 articles showed a limited diagnostic performance of serum CA-125 in detecting endometriosis (Mol et al., 1998). However, only a limited number of the included studies had a sufficient number of patients to set a cutoff value (Barbieri et al., 1986; Pittaway and Fayez, 1986; Fedele et al., 1989; Koninckx et al., 1996; Medl et al., 1997). These larger studies confirmed an elevation of serum CA-125 level in endometriosis, including both mild and severe stages, but not in mild endometriosis without ovarian involvement.

In order to evaluate the diagnostic value of CA-125 assays for possible cases of mild endometriosis, we measured serum CA-125 levels for 775 consecutive women undergoing laparotomy or laparoscopy; we analyzed results separately for
women with mild disease without ovarian endometriomas and women with severe disease with endometriomas. We also did separate analyses based on association of endometriosis with adenomyosis and leiomyomas, both of which might affect serum CA-125 level.

Materials and methods

Of consecutive patients undergoing laparoscopy or laparotomy at the Department of Obstetrics and Gynecology, Kyoto Prefectural University of Medicine, and referring hospitals in the Kyoto area between January 1999 and December 2003, all women who were diagnosed with endometriosis, adenomyosis and/or leiomyomas, or a normal pelvis were enrolled in the study. All patients were Japanese, of reproductive age, and had cyclic menstruation patterns. No patient had received endocrine therapy, including GnRH agonists, danazol, or combination estrogen–progestin therapy for at least six months before enrollment in the study. Patients were also excluded if their diagnoses included other uterine neoplasms, ovarian neoplasms, pelvic inflammation, or pregnancy. A total of 775 cases met criteria for enrollment (Table I). In cases of hysterectomy (n = 531), diagnosis of endometriosis, adenomyosis and/or leiomyomas was made on both macroscopic examination and histology of at least three sections of excised uterus. For each case in whom hysterectomy was not performed (n = 244), diagnosis was made on both macroscopic and histologic examination of excised or biopsied tissues referencing preoperative transvaginal ultrasonography and magnetic resonance imaging. Although adenomyosis and leiomyomas are separate entities, they are often found in association with each other. It is difficult to separately diagnose adenomyosis and leiomyomas; there may be a higher likelihood of overlooking the presence of the other disease. We have therefore put adenomyosis and leiomyomas in one category. Stage of endometriosis was assigned according to the revised American Society for Reproductive Medicine scoring system (American Society for Reproductive Medicine, 1997). The study protocol was approved by the Kyoto Prefectural University of Medicine institutional review board, and informed consent was obtained from each patient before enrollment.

Enrolled patients were subgrouped into women who had endometriosis only (Group E), women who had endometriosis associated with adenomyosis and/or leiomyomas (Group E/A/L), those who had adenomyosis and/or leiomyomas but not endometriosis (Group A/L), and women with a normal pelvis. Groups E and E/A/L were further subgrouped by disease involvement into endometriosis with or without ovarian endometriomas. Of the patients in groups E and E/A/L without endometriomas, 74 of 88 cases (84.0%) and 48 of 62 cases (77.4%), respectively, had early-stage (stage I or II) endometriosis. Of the patients in groups E and E/A/L with endometriomas, 153 of 161 cases (95.0%) and 111 of 122 cases (91.0%), respectively, had advanced-stage (stage III or IV) endometriosis.

Blood samples were drawn before surgery on days other than those during menstruation. Serum concentrations of CA-125 were measured by an immunoradiometric assay kit using the specific monoclonal antibody M11 (Centocor, Malvern, PA, USA) and were expressed in arbitrary units based on a primary standard. The intra- and inter-assay coefficients of variation were 3.4% at 1.4 U/mL and 5.3% at 40.7 U/mL, respectively.

Statistical analyses were performed with Statflex version 5.0 software (Artech, Osaka, Japan). The difference in serum CA-125 levels between disease groups was analyzed by the Kruskal-Wallis test followed by nonparametric Dunn’s test. Group abbreviations: E, endometriosis; E/A/L, endometriosis associated with adenomyosis and/or leiomyomas; A/L, adenomyosis and/or leiomyomas.

Table I lists the serum levels of CA-125 determined prior to surgery in the six groups with a total of 775 patients. In the 101 cases with a normal pelvis, serum CA-125 level was less than 20 U/mL in 85 cases (84.2%), less than 26 U/mL in 92 cases (91.1%), and less than 30 U/mL in 98 cases (97.0%). Only one case had a serum CA-125 level greater than the 35 U/mL value that had been initially set as an empirical cutoff level for the top of the normal range (Figure 1).

The mean serum CA-125 level in each of the five disease groups was significantly greater than that in the group with

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>Serum CA-125 level (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Normal pelvis</td>
<td>101</td>
<td>14.8 ± 6.7</td>
</tr>
<tr>
<td>E</td>
<td>249</td>
<td>72.8 ± 110.9</td>
</tr>
<tr>
<td>With endometriomas</td>
<td>88</td>
<td>50.8 ± 68.6</td>
</tr>
<tr>
<td>Without endometriomas</td>
<td>161</td>
<td>84.8 ± 126.9</td>
</tr>
<tr>
<td>E/A/L</td>
<td>184</td>
<td>70.8 ± 79.4</td>
</tr>
<tr>
<td>With endometriomas</td>
<td>62</td>
<td>60.5 ± 88.6</td>
</tr>
<tr>
<td>Without endometriomas</td>
<td>122</td>
<td>76.0 ± 74.1</td>
</tr>
<tr>
<td>A/L</td>
<td>241</td>
<td>40.8 ± 58.2</td>
</tr>
<tr>
<td>Total</td>
<td>775</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.001 versus normal pelvis.

*p < 0.001 and

*p < 0.005 versus E patients without endometriomas.

*p < 0.000 versus A/L.

Multiple comparisons were performed by the Kruskal-Wallis test followed by nonparametric Dunn’s test.

Results

Table I lists the serum levels of CA-125 determined prior to surgery in the six groups with a total of 775 patients. In the 101 cases with a normal pelvis, serum CA-125 level was less than 20 U/mL in 85 cases (84.2%), less than 26 U/mL in 92 cases (91.1%), and less than 30 U/mL in 98 cases (97.0%). Only one case had a serum CA-125 level greater than the 35 U/mL value that had been initially set as an empirical cutoff level for the top of the normal range (Figure 1).

The mean serum CA-125 level in each of the five disease groups was significantly greater than that in the group with
a normal pelvis ($P < 0.001$). The mean serum CA-125 level in E patients with ovarian endometriomas was significantly greater than that in E patients without endometriomas ($P < 0.001$). The mean level in E/A/L patients without endometriomas ($P < 0.005$) and with endometriomas ($P < 0.001$) was greater than that in E patients without endometriomas. The mean level in both E and E/A/L patients with endometriomas was greater than that in A/L patients (Table I). However, serum CA-125 levels varied widely in each disease group (Table I, Figure 1). It should be noted that some cases associated with endometriomas and with severe endometriosis had very low CA-125 levels. In A/L patients, while most of the cases associated predominantly with leiomyomas showed low levels, the cases associated predominantly with adenomyosis showed high levels despite the absence of endometriosis.

The ROC curve analysis revealed that the areas under the curves for both groups with endometriomas (E and E/A/L) were greater than 0.9, indicating a good diagnostic performance (Figure 2, Table II). However, the area under the curve for E without endometriomas was 0.788, significantly smaller than that for E with endometriomas ($P < 0.05$). The area for A/L was significantly smaller than those for E with endometriomas and E/A/L with endometriomas ($P < 0.001$) (Figure 2, Table II).

In Table II, we list three representative cutoff values that generated key values of specificity. The cutoff value 20 U/mL was the lowest level for which specificity exceeded 80%; 26 U/mL was the lowest level for which specificity exceeded 90%; and at 30 U/mL specificity reached 97%. In E without endometriomas, when the specificity was 84.2% (cutoff value, 20 U/mL) the sensitivity was only 72.7%. However, when specificity was 91.1% (cutoff value, 26 U/mL) sensitivity decreased to 45.5%, and when specificity was 97.0% (cutoff value, 30 U/mL) sensitivity was 44.3%. At the cutoff value of 20 U/mL, both maximal accuracy (78.8%) and maximal diagnostic value (61.2%) were obtained. NPV was 78.0% at the cutoff value of 20 U/mL. PPV was 92.9% at the cutoff value of 20 U/mL. Characteristically, NPVs were always lower than PPVs. For E with endometriomas and E/A/L with endometriomas, sensitivity ranged from 76.2% to 89.4%.

**Discussion**

To our knowledge, the present study is the first to analyze the usefulness of CA-125 in diagnosis of endometriosis without endometriomas. Currently, the gold standard for diagnosis of endometriosis is inspection of the abdominal cavity and histological demonstration of lesions using laparoscopy or laparotomy. However, to screen a large number of women, a less invasive, less expensive, simple, and rapid test is needed even if it is not completely accurate.

In practice, severe endometriosis can be diagnosed with high accuracy by pelvic examination. Transvaginal ultrasonography is routinely used, and it detects severe endometriosis associated with ovarian endometriomas with both high sensitivity and specificity (Guerriero et al., 1998; Patel et al., 1999; Dessole et al., 2003). Magnetic resonance imaging also detects endometriomas with high accuracy (Takahashi et al., 1994; Bis et al., 1997; Kinkel et al., 1999; Stratton et al., 2003). Adenomyosis and leiomyomas can be detected with high accuracy by pelvic examination and imaging tools (Eskenazi et al., 2001). However, these imaging tools are still not sensitive enough to detect mild endometriosis associated with peritoneal implants but not with endometriomas. Given such a situation, a serological test is most useful if it is applicable to detect endometriosis without endometriomas. CA-125 in the systemic circulation originates mostly from ectopic endometriotic tissues and, to a lesser extent,
from eutopic endometrium and ovary. However, immunohistochemical staining intensity does not correlate with the serum level (Toki et al., 2000). Although a number of studies have investigated the usefulness of serum testing for CA-125, most of these studies analyzed diagnostic performance combining mild and severe endometriosis (Barbieri et al., 1986; Pittaway and Fayez, 1986; Fedele et al., 1989; Koninckx et al., 1996; Medl et al., 1997; Cheng et al., 2002). There has been no study discriminating endometriosis with or without endometriomas. It can be estimated that the studies would have obtained a lower diagnostic value if the cases had been limited to mild endometriosis. Mol et al. (1998) reported a meta-analysis based on 23 articles; they analyzed the diagnostic performance of serum CA-125 measurement for mild plus severe endometriosis and severe endometriosis only. As expected, they obtained a better diagnostic value for severe endometriosis than combined with mild endometriosis. In diagnosing all grades of endometriosis at a specificity of 90%, sensitivity was only 28%. If sensitivity were increased to 50%, specificity dropped to 72%. In diagnosis of severe endometriosis with a specificity of 89%, sensitivity increased to 47%, and if sensitivity were increased to 60%, specificity increased to 81%.

In the present study, the mean serum CA-125 level increased when cases were associated with ovarian endometriomas. Serum CA-125 level increased with grade of endometriosis, consistent with findings from most previous studies (Barbieri et al., 1986; Pittaway and Fayez, 1986; Fedele et al., 1989; Koninckx et al., 1996; Medl et al., 1997; Cheng et al., 2002). As expected, a good diagnostic performance was obtained for endometriosis with endometriomas. However, it should be noted that as many as 10.6% of cases of E with endometriomas and 15.6% of cases of E/A/L with endometriomas had serum CA-125 levels less than 20 U/mL. In contrast, the area under the ROC curve for E without endometriomas was 0.788, indicating that the diagnostic accuracy for endometriosis without endometriomas was naturally less than that for endometriosis with endometriomas. The present study provided sufficiently high specificity but insufficiently low sensitivity compared with reports in other studies (Barbieri et al., 1986; Pittaway and Fayez, 1986; Fedele et al., 1989; Koninckx et al., 1996; Medl et al., 1997; Mol et al., 1998; Cheng et al., 2002). Accordingly, NPV was always lower than PPV.

Our population was one of the largest series comprising consecutive patients among the studies that have analyzed CA-125 levels and diagnosis of endometriosis. This might have resulted in reducing selection bias and minimizing errors in statistical values. Because PPV and NPV are influenced by the frequency of disease, this type of study offers an advantage for assessing diagnostic performance.

Given the results, although the cutoff value 20 U/mL showed the best diagnostic performance, we propose a combined use of two cutoff values, 20 and 30 U/mL, rather than a single cutoff value in diagnosis of endometriosis without endometriomas. When focused on group E without endometriomas, if a serum CA-125 level equals or is higher than 30 U/mL, the probability of endometriosis is 92.9% (PPV). However, even if a level is less than 30 U/mL, the possibility of endometriosis cannot be neglected. By contrast, if a CA-125 level is less than 20 U/mL, endometriosis can be ruled out with a probability of 78.0% (NPV). If CA-125 level equals or is higher than 20 U/mL and less than 30 U/mL, diagnostic performance is limited. This setting for cutoff values is clearly superior to the single empirical cutoff value of 35 U/mL. However, the accuracy of using only CA-125 testing for diagnosis is still limited. In clinical practice, 93% of women with a serum CA-125 level of 30 U/mL or higher who have had the diagnoses of adenomyosis, leiomyomas, ovarian tumors, pelvic inflammation, and/or pregnancy ruled out will be shown to have endometriosis on further diagnostic testing. At the lower end of possible cutoff values, 22% of women with a serum CA-125 level lower than 20 U/mL in the same diagnostic situation will still be shown to have endometriosis.

In conclusion, research to date indicates that serum CA-125 testing appears to be the least invasive, least expensive, simplest, and most rapid method to screen for the diagnosis of endometriosis. This test can be positioned for initial screening together with transvaginal ultrasonography. In the diagnosis of endometriosis without endometriomas, combined use of two cutoff values for CA-125, 20 and 30 U/mL, provides improved diagnostic performance. On the other hand, the present results also show the limit of serum CA-125 testing. Several less invasive tests have been postulated (e.g.

### Table II. Diagnostic performance of CA-125 in patients with endometriosis, adenomyosis and/or leiomyomas

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Area under ROC curve</th>
<th>Cutoff value 20 U/ml</th>
<th>Cutoff value 26 U/ml</th>
<th>Cutoff value 30 U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity (%)</td>
<td>Specificity 84.2%</td>
<td>PPV (%)</td>
<td>NPV (%)</td>
</tr>
<tr>
<td>E</td>
<td>Without endometriomas</td>
<td>0.788&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.7</td>
<td>80.0</td>
</tr>
<tr>
<td></td>
<td>With endometriomas</td>
<td>0.935&lt;sup&gt;c&lt;/sup&gt;</td>
<td>89.4</td>
<td>90.0</td>
</tr>
<tr>
<td>E/A/L</td>
<td>Without endometriomas</td>
<td>0.797</td>
<td>64.5</td>
<td>71.4</td>
</tr>
<tr>
<td></td>
<td>With endometriomas</td>
<td>0.905&lt;sup&gt;c&lt;/sup&gt;</td>
<td>84.4</td>
<td>86.6</td>
</tr>
<tr>
<td>A/L</td>
<td>0.703</td>
<td>51.9</td>
<td>88.7</td>
<td>42.3</td>
</tr>
</tbody>
</table>

<sup>a</sup><sup>p</sup> < 0.05 versus E patients with endometriomas,  
<sup>b</sup><sup>p</sup> < 0.05 versus A/L, and  
<sup>c</sup><sup>p</sup> < 0.001 versus A/L.
testing aromatase (Kitawaki et al., 1999) and leukocyte subsets (Gagne et al., 2003) for use in biopsied specimens of eutopic endometrium, and these techniques look promising. These moderately invasive techniques can be positioned as secondary tests before laparoscopy. Such systematic testing will provide more accurate diagnosis of endometriosis through a series of increasingly invasive tests.

Acknowledgements
This study was supported in part by Grants-in-Aid for Scientific Research (15591772, 15790903 and 16790965) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References


Submitted on November 5, 2004; resubmitted on February 18, 2005; accepted on February 28, 2005

CA-125 in endometriosis without endometriomas