Relevance of CA-125 in the evaluation of endometriosis

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Summary: To evaluate the clinical utility of CA-125 in the diagnosis of endometriosis and to compare the sensitivity of the serum and the peritoneal test as indicators of disease, the quantitative determination of the antigen in serum and in peritoneal fluid was performed by the IRMA-mat "two-step method", a two-site immunoradiometric assay. A total of 28 women undergoing diagnostic or operative laparoscopy with endometriosis stage I-II (10 patients), endometriosis stage III-IV (8 patients) or other benign gynecological diseases (10 patients) were studied. The results were compared with a sample of 12 women with a normal pelvis (control group). CA-125 levels in the peritoneal fluid were higher than those found in the serum and were significantly elevated ($p < 0.001$) when compared with the control group, both in women with endometriosis stage I-II and stage III-IV. In the serum CA-125 levels increased only in advanced stages of endometriosis. Levels of CA-125 in peritoneal fluid seemed to be a more sensitive indicator of disease than the serum test (0.88 vs 0.44), especially in early stage endometriosis (0.80 vs 0.20) which tends to be overlooked by the CA-125 serum test.

Key words: CA-125; Endometriosis; Infertility; Laparoscopy; Peritoneal fluid; Immunoradiometric assay.

INTRODUCTION

CA-125 is an high molecular weight antigenic determinant expressed on the surface of the coelomic epithelium, including the epithelium of the endocervix, endometrium, fallopian tube, peritoneum, pleura, pericardium, and placental tissues. Besides its presence in those tissue, CA-125 is well-known to be elevated in biological fluids such as human milk, amniotic and peritoneal fluids, cervical mucus, saliva and seminal plasma. Up to 1986, the clinical use of this antibody has been limited to the detection and management of ovarian and endometrial advanced cancer. Subsequently, many studies have found serum concentrations of CA-125, that in healthy blood donors are $\leqslant 35$ U/ml, exhibited a variable expression in women during the normal menstrual cycle, with a peak during menses. An increase in CA-125 has also been detected in other nonmalignant conditions such as pelvic inflammatory disease (PID), adenomyosis, early pregnancy and especially in advanced endo-

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metriosis ([1-7]). The origin of this increase is suggested to be endometrial because the endometrium clearly expresses CA-125 ([8]). Recently, several teams also evaluated CA-125 levels in peritoneal fluid (PF), but they found conflicting results, probably due to different assay methods ([1, 9-11]).

The aim of the present study was to determine the clinical utility of CA-125 in diagnosing endometriosis. First, we investigated the presence of CA-125 in serum and PF. Next we examined if differences in CA-125 concentrations could distinguish women with and without endometriosis. Finally, we sought evidence of a correlation between CA-125 concentrations and severity of endometriosis.

MATERIALS AND METHODS

CA-125 serum and peritoneal levels were evaluated in 28 women undergoing laparoscopy. At surgery the diagnosis was recorded and 18 infertile women found to have endometriosis were staged according to the American Fertility Society revised classification (group 1: stage I-II 10 patients; group 2: stage III-IV 8 patients). The remaining 10 patients were found to have benign gynecological conditions other than endometriosis. Diagnosis of this group included pelvic adhesions without endometriosis (4), leiomyoma (3), ovarian cysts (2), and a paratubal cyst (1). The results were compared with a sample of 12 women suffering from primary infertility who had a completely normal pelvis at laparoscopy. Every laparoscopy was performed between the 7th and the 10th day of the cycle. Blood samples were always drawn by venipuncture from the subjects immediately before the induction of general anesthesia. Peritoneal fluid samples were always aspirated at the beginning of laparoscopy taking care to avoid blood contamination.

The quantitative determination of CA-125 in serum and in peritoneal fluid was performed using IRMA-mat CA-125 "two-step method" (Byk-Stangtee Diagnostic GmbH & Co. Kgy, Dietzenbach), a two-site immunoradiometric assay (sandwich principle): the monoclonal antibody M 11 is used for the coating of the solid phase (coated tube), the monoclonal antibody OC 125 is used for the tracer. During the first incubation, the CA-125 present in patient samples and standards is bound to the antibody immobilised at the test tube wall. Unbound material is removed by a washing step. During the ensuing second incubation, the tracer antibody reacts with the CA-125 already bound. After removal of excess tracer by a second washing step, the radioactivity remaining at the tube wall is measured in a gamma scintillation counter. The reproducibility of the method is good – the intra-assay and interassay variation (CV%) were 4.3% and 7.7%, respectively.

The clinical utility of serum and peritoneal CA-125 in the diagnosis of endometriosis was evaluated by comparing sensitivity and specificity by using a cutoff of 35 U/ml for serum measurements ([2, 12]) and 60 U/ml for the peritoneal test ([13]). Sensitivity was defined as the number of patients with all stages of endometriosis who had tumor-associated antigen (TAA) levels > cut-off, divided by the total number of patients with endometriosis ([14]). Specificity was defined as the number of patients who did not have endometriosis and had TAA levels < cut-off divided by the total number of patients without endometriosis ([14]). Statistical analysis was carried out by using Student's *t*-test. Probability values < 0.01 were considered significant.

Results are reported as means ± standard deviation (SD).

RESULTS

The concentrations of CA-125 in PF were always significantly higher than the corresponding serum in all the groups (stage I-II, stage III-IV, total endometriosis).

As shown in table 1, the mean serum CA-125 levels (22.34 ± 22.51) in group 1 were not statistically different from the controls (18.71 ± 5.56), nevertheless lower than the cutoff value (35 U/ml). Patients in group 2 had significantly higher CA-125 levels (60.29 ± 23.52) when compared with the normal pelvis group ($p < 0.001$). In patients with benign gynecologic disease increase of serum CA-125 was observed in only one patient.

Peritoneal CA-125 levels both in group 1 (183.3 ± 110.72) and group 2 (408.59 ± 184.74) appeared significantly higher than the controls (57.17 ± 19.23) ($p < 0.001$).

Table 1. — *Serum and peritoneal CA-125 levels.*

	Age (years)	Serum CA-125 (U/ml)	LP CA-125 (U/ml)
Total Endometriosis . . .	31.2 ± 4.5	39.20 ± 29.54 **	283.43 ± 183.89 **
Group 1 (stage I-II) . .	30.8 ± 4.4	22.34 ± 22.51	183.30 ± 110.720 * **
Group 2 (stage III-IV) .	31.9 ± 4.7	60.298 ± 23.52 * **	408.59 ± 184.74 * **
Benign gynecologic disease .	32.6 ± 6.1	19.86 ± 9.38	67.97 ± 55.51
Controls	27.0 ± 5.8	18.71 ± 5.56	57.17 ± 19.23

* $P < 0.001$ compared with the control group;
** $P < 0.001$ compared with the benign gynecological disease group.

Eight out of 10 patients with endometriosis stage I-II had increased PF concentrations of CA-125, whereas all the patients with endometriosis stage III-IV showed increased levels. In patients with benign gynecological disease 4 out of 10 patients displayed increased CA-125 peritoneal concentrations, and the PF levels in this group were significantly different when compared with the patients with endometriosis.

The sensitivity and specificity are reported in table 2.

DISCUSSION

Many investigators ([6, 15-19]) have studied the value of CA-125 as a diagnostic test for endometriosis. Serum CA-125 concentrations are directly caused by endometriotic lesions shedding cell-surface antigens into the systemic circulation ([2]). The values found in the present study are in agreement with those reported in the literature. A significant difference was seen only between patients with endometriosis stage III-IV and the controls ($p < 0.001$), whereas women with early stage endometriosis had low levels which are not statistically significant.

Indeed, in women with advanced stages of endometriosis the peritoneum is damaged at the sites of endometriotic implants, resulting in an higher release of CA-125 antigen into the circulation ([9, 20]). The sensitivity of CA-125 serum measurement is too low (0.44) to use as a screening or diagnostic test for endometriosis. Nevertheless, many authors have shown that the test is helpful in monitoring the progress of patients after medical or surgical therapy ([4, 12, 21-23]), and that an elevated plasma CA-125 concentration should be investigated as an indication for laparoscopy in women with chronic pelvic pain, even in the absence of clinical signs of endometriosis ([10]).

Table 2. — *Reliability of Serum * and Peritoneal ** CA-125 levels for the Diagnosis of Endometriosis.*

	Serum			Peritoneal Fluid		
	Tot.	Stage I-II	Stage III-IV	Tot.	Stage I-II	Stage III-IV
Sensitivity	0.44	0.20	0.75	0.88	0.80	1
Specificity	0.9	—	—	0.5	—	—

* CA-125 > 35 U/ml; ** CA-125 > 60 U/ml.

In peritoneal fluid, a significant difference ($p < 0.001$) was found between both group 1 and 2 and the controls Our results showed that CA-125 levels in PF appear to be a more sensitive indicator of disease than serum concentrations (0.88 vs 0.44), mainly in stage I-II (0.80 vs 0.20) which tends to be overlooked by the CA-125 serum test. Similar results led Barbati, *et al.* ([9]) to attempt a clinical use of CA-125 in PF aspirated by culdocentesis. The test can be helpful in screening women at risk of undergoing laparoscopy. To date, the utility of CA-125 in the detection of early stage endometriosis is however limited because of its low specificity. Indeed in benign gynecological disease the CA-125 levels in PF were elevated in 40% of patients (4 out of 10), whereas in their respective serum, moderate increased values were detectable only in 10% of the cases (1 out of 10). Nevertheless, the CA-125 test could be helpful in monitoring the effectiveness of treatment in women with endometriosis stage I-II.

In conclusion, further investigations are needed to verify the sensitivity of serum and peritoneal CA-125 as diagnostic tests for endometriosis: using cutoff levels lower for serum ([11]) and higher for PF, or different assays with high dilutions of the samples ([9]). According to the hypothesis that endometriosis implants secrete cytokines that recruit and activate peritoneal macrophages ([24]), we are trying to use others markers (IL-5, IL-6, IL-8) to verify the reliability of a single serum and/or peritoneal drawing for the diagnosis of endometriosis.

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