Prognostic value of serum CA-125 measurements on stimulation day 1 and on the day of oocyte pickup in the prediction of IVF treatment outcome

Erik E. Hauzman,^{1,3} Ana R. Lagarde,² Katalin Nagy,² Péter Fancsovits,¹
Ákos Murber,¹ Gyöző Jánoki,² Zoltán Papp,¹ and János Urbancsek¹

Submitted October 15, 2004; accepted March 14, 2005

Purpose: To assess whether serum levels of CA-125, a proposed marker of endometrial receptivity, are associated with pregnancy after IVF.

Methods: 42 IVF pregnancies were matched with nonpregnant controls. Twenty-eight additional unmatched pregnancies were included for the comparison of pregnancy outcomes. CA-125 concentrations were measured on stimulation day 1 (SD1) and at oocyte pickup (OPU). We analyzed association between CA-125 levels and establishment of pregnancy and its outcome; correlation between CA-125 concentrations and other parameters; differences in CA-125 levels between SD1 and OPU.

Results: In pregnant, but not in nonpregnant patients, CA-125 levels decreased significantly from SD1 to OPU. There was no significant difference in CA-125 levels at either time point between pregnant and nonpregnant subjects. No significant correlation was found between CA-125 levels and any other clinical or laboratory parameters.

Conclusions: CA-125 measurements at the beginning of stimulation or at oocyte retrieval are not useful in predicting IVF outcome.

KEY WORDS: CA-125; inhibin B; in vitro fertilization; pregnancy outcome.

INTRODUCTION

The majority of failures following in vitro fertilization (IVF) occur after embryo transfer (ET). Apart from abnormal embryos, a nonreceptive endometrial environment may also be responsible for implantation failure (1). Therefore, a marker for the prediction of endometrial receptivity would be of great use in deciding whether to proceed to ET or to cryopreserve the embryos.

Assessment of endometrial receptivity can be based on histologic dating of the endometrium (2), evaluation of endometrial thickness and blood flow by transvaginal ultrasound (US) with color Doppler (3–5), and on the measurement of uterine secretory proteins, including CA-125, which has been studied most extensively.

CA-125, an antigenic determinant of a glycoprotein expressed by most epithelial ovarian cancers, can also be measured in the peripheral circulation of healthy females (6). However, contradictory data have been published on its exact source, whether endometrium or ovary. Studies on the value of CA-125 levels in the prediction of pregnancies, as measured at different time points of IVF treatment (5,7–9), have also yielded inconclusive data. Therefore, we examined whether serum CA-125 levels at oocyte pickup or on the first

¹ First Department of Obstetrics and Gynecology, Semmelweis University, Faculty of Medicine, Budapest, Hungary.

² “Fodor József” National Center of Public Health, “Frédéric Joliot-Curie” National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary.

³ To whom correspondence should be addressed; e-mail: humanic@noi1.sote.hu.

day of ovarian stimulation have any value in the prediction of treatment outcome and tried to infer consequences as to its source.

MATERIALS AND METHODS

Forty-two consecutive IVF patients with a clinical pregnancy (pregnant group) were matched with patients in whom treatment did not result in pregnancy (nonpregnant group) by age, type of infertility, serum estradiol (E_2) levels at ovulation induction, number of oocytes retrieved, and number of embryos replaced. Clinical pregnancy was detected if a fetal heartbeat was visible on US at 6–7 weeks of gestation. Twenty-eight additional consecutive pregnant patients without matched pairs were included to compare different pregnancy outcomes. Since CA-125 concentrations are known to be elevated in endometriosis (10), no endometriosis patients were included.

Ovarian stimulation, ovulation induction, and oocyte pickup were carried out as described elsewhere (11). Retrieved oocytes were fertilized with either IVF or ICSI technique. Up to four embryos were replaced 2 or 3 days later.

Venous blood samples were taken in the early follicular phase of a spontaneous cycle and assayed for serum FSH and E_2 . Estradiol was also measured in morning samples collected immediately before starting gonadotropin administration (day 1) and on the day of ovulation induction. CA-125 and inhibin B measurements were performed in serum samples collected on day 1 and on the day of oocyte pickup.

The description of FSH, E_2 , and inhibin B assays can be found elsewhere (12). CA-125 concentrations were determined using an immunoradiometric kit ("Fodor József" National Center of Public Health, "Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary, in cooperation with CIS bio international, Gif-sur-Yvette, France) (detection limit, 0.5 U/mL, intra- and inter assay coefficients of variation, <4% and <5% over the range 0–750 U/mL).

Pregnancies were classified as early pregnancy loss [first-trimester abortions ($n = 16$) and ectopic pregnancies ($n = 3$)] and ongoing pregnancies, i.e., progression beyond 12 weeks' gestation [singleton ($n = 27$) and multiple deliveries ($n = 21$), and second-trimester abortions ($n = 3$)]. No preclinical abortions were included.

McNemar's test, t -test for dependent or independent samples, Wilcoxon's matched pairs signed rank sum test, the Mann–Whitney U test, and Pearson's product-moment correlation analysis (r) was performed where appropriate. Power analysis showed that a 10 U/mL difference in CA-125 levels could be detected with nearly 100% power in our sample. All tests were two-tailed, with a confidence level of 95% ($p < 0.05$).

RESULTS

Baseline and stimulation characteristics were similar in pregnant and nonpregnant patients (Table I). No significant difference was observed, as expected, in E_2 concentrations on the day of ovulation induction. Neither E_2 nor CA-125 or inhibin B before start of stimulation differed significantly between pregnant and nonpregnant subjects. No significant difference was observed in CA-125 or inhibin B, concentrations on the day of oocyte pickup either.

No statistically significant correlation was found between CA-125 levels and same-day E_2 or inhibin B concentrations, nor did CA-125 concentrations significantly correlate with E_2 levels at ovulation induction or the number of oocytes retrieved (data not shown).

Table I. Clinical and Endocrine Characteristics of Pregnant and Nonpregnant Patients

	Pregnant group	Nonpregnant group	p
Age (years)	31.1 $\pm$ 3.8	30.9 $\pm$ 3.5	ns
BMI (kg/m ²)	23.1 $\pm$ 3.2	22.4 $\pm$ 3.2	ns
Main infertility factor	13/26/3	8/32/2	ns
(male/tubal/unexplained)			
Treatment (IVF/ICSI)	31/11	35/7	ns
FSH basal (IU/L)	6.7 $\pm$ 1.7	6.5 $\pm$ 1.8	ns
E_2 basal (pg/mL)	47.1 $\pm$ 20.1	44.6 $\pm$ 18.5	ns
Days of stimulation	10.8 $\pm$ 1.4	10.3 $\pm$ 1.2	ns
Ampoules of gonadotropins	30.5 $\pm$ 15.5	25.9 $\pm$ 8.9	ns
E_2 on day 1 of stimulation	37.7 $\pm$ 13.6	40.0 $\pm$ 20.7	ns
(pg/mL)			
Inhibin B on day 1 of stimulation	24.2 $\pm$ 20.1	29.5 $\pm$ 16.6	ns
(pg/mL)			
CA-125 on day 1 of stimulation	29.9 $\pm$ 21.1	26.3 $\pm$ 15.5	ns
(U/mL)			
E_2 on day of OI (pg/mL)	2499 $\pm$ 1099	2475 $\pm$ 1217	ns
Inhibin B on day of OPU (pg/mL)	299 $\pm$ 191	242 $\pm$ 152	ns
CA-125 on day of OPU (U/mL)	22.2 $\pm$ 12.9	18.7 $\pm$ 11.0	ns
No. of oocytes retrieved	7.3 $\pm$ 2.3	7.3 $\pm$ 2.4	ns
No. of embryos replaced	3.5 $\pm$ 0.7	3.5 $\pm$ 0.7	ns

Note. Data are mean $\pm$ SD, all statistical tests are paired, BMI: body mass index; OI: ovulation induction; OPU: oocyte pickup; ns: not significant.

Within pregnant cycles, patients with early pregnancy loss ($n = 19$) had lower CA-125 levels on the day of oocyte retrieval (mean $\pm$ SD, 25.4 ± 18.7 U/mL) than patients with ongoing pregnancy ($n = 51$; 31.5 ± 19.9 U/mL) but this difference was just above the selected level of statistical significance ($p = 0.044$). Within the latter group, singleton ($n = 29$) and multiple ongoing pregnancies ($n = 22$) were compared and found to have similar serum concentrations of CA-125 at oocyte pickup (26.0 ± 14.6 vs. 37.6 ± 23.9 U/mL). No significant difference was observed between any pregnancy subgroups in CA-125 levels on day 1 of ovarian stimulation (data not shown).

In pregnant patients with serum samples available from both stimulation day 1 and day of oocyte pickup ($n = 15$), CA-125 levels were significantly lower at oocyte collection than on day 1 ($p = 0.016$) (Table I and Fig. 1). No such difference was observed among nonpregnant patients ($n = 14$; $p = 0.18$).

DISCUSSION

Previous reports on the prognostic value of CA-125 in IVF cycles yielded contradictory results. Lanzzone *et al.* found constant serum concentrations throughout stimulation and concluded that CA-125 was not useful in the prediction of treatment outcome (13). In a study conducted by Miller *et al.*, higher CA-125 levels were observed in pregnant than in nonpregnant cycles on the day before and, even more markedly, on the day of ovu-

lation induction (7). Chryssikopoulos *et al.* found comparable serum concentrations on the day of ovulation induction but significantly higher values were observed at oocyte retrieval in the pregnant group (8). This difference was lost, however, on the day of ET. Positive results were also reported by Tavmergen *et al.* (14), whereas Brandenberger *et al.* demonstrated similar concentrations in conception and nonconception cycles for all sampling days mentioned above (15). In a prospective study of 71 infertile women, substantial fluctuations were observed during ovarian suppression and stimulation as well as in the luteal phase, but these changes were independent of the outcome of IVF treatment (16). In contrast with findings mentioned so far, Noci *et al.* found significantly lower CA-125 concentrations in pregnant than in nonpregnant patients at oocyte pickup (9).

We observed no correlation between serum CA-125 and inhibin B concentrations measured in the same sample or with E_2 levels at ovulation induction or the number of oocytes retrieved, which argues against an ovarian source of serum CA-125 during stimulation (7,14,17). A further counterargument is that CA-125 could not be detected immunohistochemically in theca or granulosa cells (6), and no production could be demonstrated by human granulosa cells in culture (17).

In earlier studies, no correlation with endometrial thickness or vascularization was observed (5,7), which argues against an endometrial source. However, a functional rather than quantitative change of

Fig. 1. Changes in serum CA-125 levels from stimulation day 1 (SD1) to day of oocyte pickup (OPU) in pregnant (A) and nonpregnant patients (B). All individual cases are depicted separately.

the endometrium still may be at the origin of CA-125 changes (7).

Observations reported in the literature agree that CA-125 levels rise during the menstrual period as a consequence of endometrial disruption (18,19). This is probably due to an easier access of CA-125 from the endometrial epithelial lining into the circulation during menstruation (20). Thus, endometrial shedding might directly be responsible for the rise of serum CA-125 levels. Noci *et al.* observed lower serum CA-125 concentrations at oocyte retrieval in pregnant than in nonpregnant cycles (9) and concluded that CA-125 might reflect the actual state of the endometrium and its tendency to shedding. In our study, no differences in CA-125 concentrations were observed between conception and nonconception cycles. Nevertheless, a decrease in serum levels by the time of oocyte pickup, as compared to the beginning of stimulation, was found in the pregnant group (Fig. 1A), whereas the change was far less uniform in nonpregnant cycles and no significant difference was observed in the latter group (Fig. 1B). Extending the idea of Noci *et al.* (9), this decrease could also suggest a more compact endometrium, which might be more receptive to the implantation of the embryo.

In summary, our data suggest that measurement of serum CA-125 levels during stimulation is not predictive of subsequent pregnancy, even though a decrease from the start of stimulation to oocyte retrieval seemed to be associated with the establishment of pregnancy. Therefore, determination of CA-125 does not appear to be useful in predicting the outcome of IVF treatment.

REFERENCES

1. Paulson RJ, Sauer MV, Lobo RA: Factors affecting embryo implantation after human in vitro fertilization: a hypothesis. *Am J Obstet Gynecol* 1990;163:2020–2023
2. Graf MJ, Reyniak JV, Battle-Mutter P, Laufer N: Histologic evaluation of the luteal phase in women following follicle aspiration for oocyte retrieval. *Fertil Steril* 1988;49:616–619
3. Gonen Y, Casper RF, Jacobson W, Blankier J: Endometrial thickness and growth during ovarian stimulation: a possible predictor of implantation in in vitro fertilization. *Fertil Steril* 1989;52:446–450
4. Steer CV, Tan SL, Dillon D, Mason BA, Campbell S: Vaginal color Doppler assessment of uterine artery impedance correlates with immunohistochemical markers of endometrial receptivity required for the implantation of an embryo. *Fertil Steril* 1995;63:101–108
5. Vujisić S, Kupešić S, Mihaljević D, Akšamija A, Kurjak A: Evaluation of serum CA 125 concentration before and during hormonal induced cycles as predictor of IVF/ET outcome. *Am J Reprod Immunol* 2002;48:355–360
6. Zeimet AG, Müller-Holzner E, Marth C, Daxenbichler G, Dapunt O: Tumor marker CA-125 in tissues of the female reproductive tract and in serum during the normal menstrual cycle. *Fertil Steril* 1993;59:1028–1035
7. Miller KA, Deaton JL, Pittaway DE: Evaluation of serum CA 125 concentrations as predictors of pregnancy with human in vitro fertilization. *Fertil Steril* 1996;65:1184–1189
8. Chryssikopoulos A, Mantzavinos T, Kanakas N, Karagouni E, Dotsika E, Zourlas PA: Correlation of serum and follicular fluid concentrations of placental protein 14 and CA-125 in in vitro fertilization-embryo transfer patients. *Fertil Steril* 1996;66:599–603
9. Noci I, Maggi M, Biagiotti R, D'Agata A, Criscuoli L, Marchionni M: Serum CA-125 values on the day of oocyte retrieval are not predictive of subsequent pregnancy with in-vitro fertilization. *Hum Reprod* 1999;14:1773–1776
10. Barbieri RL, Niloff JM, Bast RC, Jr., Scaetzel E, Kistner RW, Knapp RC: Elevated serum concentrations of CA-125 in patients with advanced endometriosis. *Fertil Steril* 1986;45:630–634
11. Hauzman E, Fedorcsák P, Klinga K, Papp Z, Rabe T, Strowitzki T, Urbancsek J: Use of serum inhibin A and human chorionic gonadotropin measurements to predict the outcome of in vitro fertilization pregnancies. *Fertil Steril* 2004;81:66–72
12. Urbancsek J, Hauzman E, Klinga K, Rabe T, Papp Z, Strowitzki T: Use of serum inhibin B levels at the start of ovarian stimulation and at oocyte pickup in the prediction of assisted reproduction treatment outcome. *Fertil Steril* 2005;83:341–348
13. Lanzone A, Fulghesu AM, Guida C, Muscatello R, Caruso A, Mancuso S: Serum CA-125 levels do not depend on ovarian steroidogenesis. *Fertil Steril* 1990;54:415–418
14. Tavmergen E, Sendag F, Goker EN, Levi R: Value of serum CA-125 concentrations as predictors of pregnancy in assisted reproduction cycles. *Hum Reprod* 2001;16:1129–1134
15. Brandenberger AW, Bersinger NA, Huber PR, Berger E, Glanzmann P, Birkhaeuser MH: CA-125 concentrations in the serum and pregnancy outcome in IVF cycles. *J Assist Reprod Genet* 1998;15:390–394
16. Unkila-Kallio L, Tiitinen A, Alfthan H, Vuorela P, Stenman U, Ylikorkala O: Effect of an in vitro fertilization program on serum CA 125, tumor-associated trypsin inhibitor, free beta-subunit of human chorionic gonadotropin, and common alpha-subunit of glycoprotein hormones. *Fertil Steril* 2000;74:1125–1132
17. Baalbergen A, Janssen JW, van der Weiden RM: CA-125 levels are related to the likelihood of pregnancy after in vitro fertilization and embryo transfer. *Am J Reprod Immunol* 2000;43:21–24
18. Pittaway DE, Faye JA: Serum CA-125 antigen levels increase during menses. *Am J Obstet Gynecol* 1987;156:75–76
19. Bon GG, Kenemans P, Dekker JJ, Hompes PG, Verstraeten RA, van Kamp GJ, Schoemaker J: Fluctuations in CA 125 and CA 15-3 serum concentrations during spontaneous ovulatory cycles. *Hum Reprod* 1999;14:566–570
20. Mastropaolo W, Fernandez Z, Miller EL: Pronounced increases in the concentration of an ovarian tumor marker, CA-125, in serum of a healthy subject during menstruation. *Clin Chem* 1986;32:2110–2111