

A challenge to the concept of tubal reflux to explain the rise and fall of CA125 in serum during the first trimester

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Although amniotic fluid concentrations of cancer antigen (CA) 125 rise during the first two trimesters of pregnancy, the serum concentrations of CA125 peak during the first trimester and drop to non-pregnant values in the second and third trimester. A previous hypothesis to explain this phenomenon was that in the early first trimester decidual CA125 gains access to the maternal compartment via 'tubal reflux' and subsequent absorption by peritoneal lymphatics. However, as pregnancy advances, the decidua capsularis fuses with the decidua parietalis, thus obliterating the endometrial cavity at 10-12 weeks; the Fallopian tubes thus become functionally obstructed. To test this hypothesis, we evaluated early first trimester CA125 concentrations in women conceiving by in-vitro fertilization (IVF) and embryo transfer with patent tubes (group 1) and in those conceiving by IVF and embryo transfer with bilateral tubal occlusion (group 2). We also compared those conceiving with human menopausal gonadotrophin therapy for ovulation induction without assisted reproduction (group 3) and those conceiving without fertility drugs in assisted reproduction (group 4). Mean CA125 concentrations were similar in groups 1-3; the mean CA125 concentration in group 4 was lower but this difference was not statistically significant, probably due to the small sample size. These data do not support the concept that tubal reflux explains the rise and fall of serum concentrations of CA125, since these were equal in IVF conceptions with or without tubal patency.

Key words: CA125/IVF-embryo transfer/pregnancy/tubal occlusion/tubal reflux

Introduction

The antigenic determinant cancer antigen (CA) 125 was first identified with the murine monoclonal antibody OC 125; it was initially found to be present in >80% of ovarian epithelial tumours (Bast *et al.*, 1983). The mechanism for the increased serum concentrations is thought to be related to secreting

or sloughing from the surface of malignant ovarian cells subsequently gaining access to the circulation. The neoplastic epithelium of benign and malignant serous and mucinous ovarian cysts contains a high concentration of CA125; however, there is frequently poor correlation with serum concentrations. The leading hypothesis to explain this discrepancy is that, because the CA125 molecule is of large molecular weight, it is generally excluded from the circulation by an intact epithelial basement membrane and that CA125 can gain access to the circulation only after the membrane is disrupted by infiltrating tumour cells (Fleuren *et al.*, 1987).

Amniotic fluid CA125 concentrations appear to rise during the first two trimesters of pregnancy to values approaching those found in ovarian cyst fluid (6000-10 000 IU/ml) (Fleuren *et al.*, 1987). Interestingly, however, the serum concentrations of CA125 peak during the first trimester and drop to non-pregnant values in the second and third trimesters (Niloff *et al.*, 1984; Takahashi *et al.*, 1985; Seki *et al.*, 1986). Quirk *et al.* (1988) proposed an interesting hypothesis to explain this apparent paradox. In early pregnancy, before the endometrial cavity is obliterated by the enlarging gestational sac, decidual CA125 gains access to the maternal compartment via 'tubal reflux' and subsequent absorption via peritoneal lymphatics. The hypothesis continues that as pregnancy advances and the decidua capsularis fuses with the decidua parietalis (thus obliterating the endometrial cavity at 10-12 weeks), the Fallopian tubes become functionally obstructed. As a result, CA125 is excluded from the maternal compartment and is preferentially secreted into amniotic fluid.

The study presented herein tested Quirk's hypothesis by comparing CA125 concentrations in patients conceiving with in-vitro fertilization (IVF) who had either occluded or patent tubes. Two additional control groups were studied: those conceiving without and with follicle-stimulating drugs; these groups were included to rule out the possibility that the source of CA125 in stimulated patients could be direct secretion by the overstimulated ovaries, and that this secretion could possibly obscure the differences in CA125 concentrations contributed by pregnancy with or without tubal reflux.

Materials and methods

Patient selection

Patients were eligible for this study if they conceived following therapy for infertility at a university-associated infertility practice. Patients were classified into four groups by the therapy received in the cycle in which conception occurred

and by the patency of their tubes. The groups were as follows: group 1, IVF and embryo transfer performed in patients with patent tubes ($n = 15$); group 2, IVF and embryo transfer performed in patients with bilateral tubal occlusion ($n = 15$); group 3, ovulation induction using human menopausal gonadotrophin (HMG) in women with patent tubes who conceived without assisted reproductive techniques ($n = 25$); group 4, progesterone supplementation in the luteal phase for women conceiving without the use of ovulation induction drugs or assisted reproductive techniques ($n = 25$). All patients were required to have had a laparoscopy within 2 years prior to current treatment. Patients who had endometriosis, fibroids, or serum oestradiol concentrations surpassing 2000 pg/ml were excluded from this study, since these conditions have been shown to be associated with increased serum concentrations of CA125.

Infertility therapies

The ovarian stimulation protocol used for IVF was the luteal phase leuprolide acetate/HMG protocol described previously (Meldrum *et al.*, 1989). Ovulation induction in non-assisted reproductive cycles was performed using 150 IU of HMG beginning on day 3–5 of the cycle and continuing until a mature follicle (mean diameter >18 mm in conjunction with a serum oestradiol concentration >200 pg/ml) was observed sonographically. Then, 10 000 IU of HCG was administered. Progesterone supplementation (oral micronized progesterone 50 mg four times daily) was begun in the luteal phase and continued throughout the first trimester. Patients not requiring ovulation induction followed the same protocol for progesterone supplementation in the luteal phase of the cycle of conception and in the first trimester. When pregnancy was established, the progesterone dosage was doubled and adjusted by weekly serum progesterone monitoring to keep the serum progesterone concentration ≥ 35 ng/ml.

Serum concentrations assayed

Patients were instructed to have serum drawn for β HCG and CA125 on days 18 (week 2) and 25 (week 3) from day of oocyte retrieval (groups 1 and 2) or estimated day of ovulation (groups 3 and 4). If a patient failed to return on either day 18 or 25 they were withdrawn from the study. Ovulation in group 3 was assumed to be 36 h after the HCG injection; the day of ovulation in group 4 was considered to be 36 h from urinary luteinizing hormone (LH) rise, which was assayed every 4–6 h once a mature follicle was observed by ultrasound (18–24 mm average diameter).

The CA125 assay used was a simultaneous sandwich, solid-phase radioimmunoassay system (Centocor Inc., Malvern, PA, USA). Samples were run in duplicate and the average response calculated. If the duplicates varied by $>10\%$, the sample was re-assayed. Because all of the samples from the same patient were not run in the same assay, interassay variability was assessed and found to be 11.83%.

Statistical analysis

The mean concentrations of CA125 drawn on days 18 and 25 after oocyte retrieval or ovulation were compared between the

four groups using analysis of variance, with $P < 0.05$ considered significant.

Results

The mean serum CA125 concentrations according to the treatment regimen and patency status of the Fallopian tubes are presented in Table I. The CA125 concentrations were not normally distributed; the SD differed by group. Since the means were proportional to the range in all samples, the data were transformed using a natural logarithmic transformation. The transformed data had a normal distribution and the variances were homogeneous between groups, so the assumptions of analysis of variance were met. Since data were missing for several patients, repeated measures analysis could not be done (58% of the patients had serum drawn 18 days from retrieval or ovulation and 73% had day 25 values taken). The data for days 18 and 25 were evaluated independently.

The comparison of mean log (CA125) by treatment group is presented in Table II. The analysis of variance did not detect any difference in the mean log (CA125) concentrations between the four groups. The sample size had 80% power to detect a difference of 1.2 between the mean log CA125 concentrations at the 5% level of significance.

There was no significant correlation between the β HCG and CA125 concentrations (both were transformed logarithmically). The Pearson correlations were 0.174 at day 18 and 0.049 at day 25.

Table I. Mean serum CA125 concentrations (\pm SD) according to treatment regimen and patency status of Fallopian tubes

Group	CA125 (IU/ml) week 2	CA125 (IU/ml) week 3
1. IVF, patent tubes	70.8 \pm 89.7	77.7 \pm 80.0
Range	256 (7–263)	225 (12–237)
n	11	10
2. IVF, occluded tubes	58.2 \pm 70.5	76.1 \pm 57.5
Range	233 (18–251)	171 (19–190)
n	10	7
3. HMG, no IVF	47.4 \pm 66.1	62.7 \pm 74.9
Range	212 (8–220)	311 (7–318)
n	11	22
4. No HMG, no IVF	34.2 \pm 24.7	41.6 \pm 60.7
Range	129 (2–131)	214 (6–220)
n	14	19

Table II. Comparison of mean log (CA125) by treatment group

Group	Mean log (CA125) week 2 ^a	Mean log (CA125) week 3 ^b
1. IVF, patent tubes	3.5 \pm 1.2 ($n = 11$)	3.8 \pm 1.1 ($n = 10$)
2. IVF, occluded tubes	3.7 \pm 8.0 ($n = 10$)	4.1 \pm 0.8 ($n = 7$)
3. HMG, no IVF	3.2 \pm 1.0 ($n = 11$)	3.6 \pm 1.0 ($n = 22$)
4. No HMG, no IVF	3.0 \pm 1.1 ($n = 14$)	3.1 \pm 1.0 ($n = 19$)

^a^bAnalysis of variance showed there were no significant differences between the groups in week 2 or 3.

Discussion

The analysis did not find any difference between mean CA125 concentrations in the four groups. The failure to find significance is probably due to the small sample size since the study had 80% power to detect a difference of 1.2 or greater in the mean log (CA125) concentrations. The trend exhibited by the data suggests that there is an increase in CA125 concentrations in the HMG groups compared with the group not taking follicle maturing drugs, suggesting some increase in CA125 concentrations caused by ovarian production or increased permeability of CA125 from the amniotic sac in the presence of higher serum oestradiol concentrations.

The fact that the CA125 concentrations were the same in all three groups receiving HMG, including group 2 with tubal occlusion, indicates that tubal reflux cannot be the mechanism for the first trimester increase in CA125; thus Quirk's interesting hypothesis is refuted. The similar values seen in group 3 where no retrieval was performed, compared with groups 1 and 2, indicates that follicular puncture does not contribute to the serum CA125 concentrations.

Serum CA125 concentrations may be increased in patients suffering from ovarian hyperstimulation syndrome (OHSS) (Jager *et al.*, 1987). None of our patients clinically had OHSS. We limited the study to patients whose CA125 values were not >2000 pg/ml to eliminate possible contribution from severe OHSS. The study was not designed to answer the question whether hyperstimulation causes increased CA125 concentrations by direct secretion from ovaries or if the higher oestradiol concentrations result in increased permeability from the amniotic sac to maternal circulation. A recent publication suggests that the rise in CA125 related to ovarian hyperstimulation may involve a peritoneal contribution rather than a significant involvement of corpus luteum and/or the endometrium as sources of CA125 (Ozaksit *et al.*, 1993). CA125 concentrations have also been found to be increased in patients with endometriosis and leiomyomata (Check *et al.*, 1991; Hornstein *et al.*, 1992; Jacobs *et al.*, 1988; O'Shaughnessy *et al.*, 1993). Thus patients with these conditions were excluded from the study.

These data could also be considered consistent with the conclusion that no maternal serum CA125 may be of decidual origin and may be made by the normal endometrium (Bischof *et al.*, 1986; Jacobs *et al.*, 1988). Further research into the origin of the mechanism for rise of CA125 in pregnancy may provide clinically useful information about certain pathological processes occurring when abnormal patterns of CA125 increase are exhibited during pregnancy. At present there is no clear-cut evidence that the measurement of this antigen during the first trimester will help the clinician to diagnose abnormal pregnancy states when the concentrations do not follow the normal pattern (i.e. too high or too low) (Check *et al.*, 1990a,b; Hornstein *et al.*, 1994; Brumsted *et al.*, 1990).

Unfortunately, we do not perform natural IVF cycles ourselves, but hopefully this report may interest another IVF centre performing single oocyte retrievals to compare serum CA125 concentrations in patients with occluded versus patent tubes to help corroborate our findings in the absence of the influence of ovarian stimulation.

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