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Fluctuations in CA 125 and CA 15–3 serum concentrations during spontaneous ovulatory cycles

Gijsbert G.Bon[1], Peter Kenemans[1,4], Judith J.Dekker[2], Peter G.Hompes[2], Rob A.Verstraeten[1], Gerard J.van Kamp[3] and Joop Schoemaker[2]

[1]Department of Obstetrics and Gynaecology, [2]Research Institute for Endocrinology, Reproduction and Metabolism, and [3]Department of Clinical Chemistry, Academic Hospital Vrije Universiteit, De Boelelaan 1117, NL-1081 HV Amsterdam, The Netherlands

[4]To whom correspondence should be addressed

The aim of this study was to investigate cycle dependent changes of serum CA 125 and CA 15–3 concentrations during spontaneous ovulatory cycles. Twenty apparently healthy women with spontaneous menstrual cycles attending our infertility clinic were included. Of these women, 18 had occluded tubes as a result of sterilization. Ovulation was confirmed by luteinizing hormone test and ultrasonography and, to exclude endometriosis, a laparoscopy was performed. Serum samples for CA 125, CA 15–3, 17β-oestradiol and progesterone determinations were taken every second day starting on the 2nd day of the cycle until the 7th day of the next cycle. After correction for inter-individual variation in serum concentrations, highest CA 125 concentrations were found during the menstruation. During the follicular and peri-ovulatory phase CA 125 serum concentrations were lowest. For CA 15–3, serum concentrations were not statistically different throughout the cycle. CA 125 and oestradiol concentrations were negatively correlated, CA 15–3 and oestradiol concentrations were positively correlated. Absolute serum concentrations of both CA 125 and CA 15–3 vary among females. Within the female, fluctuations of CA 125 are phase related. In the population studied most of the patients had tubal obstruction and high CA 125 serum concentrations during menstruation, which revokes the theory that the menstrual rise of CA 125 is due only to retrograde menstruation.

Key words: CA 125/CA 15–3/menstrual cycle/MUC1/polymorphic epithelial mucin

Introduction

Measurement of tumour associated antigens in serum, employing monoclonal antibody (MAb) technology, has become an important clinical tool in the management of cancer patients. In gynaecological oncology, CA 125 and CA 15–3 determinations are routinely performed for the follow-up of ovarian and breast carcinoma, respectively (for reviews see Kenemans *et al*., 1988, 1993). CA 125 antigen is expressed in all three derivates of coelomic epithelium and can mainly be demonstrated in the epithelium of the endocervix, the Fallopian tubes, on the apical surfaces of the glandular epithelium and in the secretory products of endometrial glands in proliferative and secretory endometrium (Quirk *et al*., 1988). Extraordinarily high CA 125 concentrations are present in tubal and uterine secretions, and in cervical mucus of healthy premenopausal women (de Bruijn *et al*., 1986). CA 125 serum concentrations in premenopausal women are higher than those in postmenopausal women (Bon *et al*., 1996). A possible explanation for higher mean CA 125 serum concentrations in premenopausal women are (retrograde) menstruation, occult endometriosis externa and pelvic inflammatory disease, all conditions more prevalent in premenopausal women (Kenemans *et al*., 1993).

The CA 15–3 serum assay detects a highly glycosylated *MUC1* gene-derived transmembrane molecule, also designated polymorphic epithelial mucin (PEM) which is present on and produced by normal glandular epithelial cells. Its function is suggested to be protection, lubrication and prevention of cell to cell adhesion (Hilkens *et al*., 1992). Until now, no studies have been performed to assess a possible cycle (hormone) dependency of PEM serum concentrations in healthy premenopausal women.

The objective of the present study was to investigate possible fluctuations in serum concentrations of both CA 125 and CA 15–3 in women throughout normal ovulatory cycles and to determine whether or not these antigen concentrations are related to cyclic changes of oestradiol and progesterone.

Materials and methods

Patients and sera

Twenty otherwise apparently healthy women attending our outpatient infertility clinic were included in this study (age: range 28–38 years, median 34.5 years). Eighteen of these patients were infertile because of tubal occlusion as a result of sterilization, two because of male infertility. All women had regular spontaneous ovulatory cycles and were not taking any medication at the time of blood sampling. Cycles were subdivided according to the luteinizing hormone (LH) peak and menstrual period into menstrual phase I (M1, actual bleeding during the beginning of the cycle), follicular phase (F), peri-ovulatory phase (PO) (cycle day –2 to +2 days to LH peak), luteal phase (L) and menstrual phase II (M2, actual bleeding of next cycle). In all women, the moment of ovulation was confirmed by LH test and transvaginal ultrasound and an endometrial biopsy was taken during the luteal phase to exclude luteal insufficiency. Each patient underwent a diagnostic laparoscopy in which endometriosis was excluded. Cycle duration ranged from 23–32 days (median: 28 days). A total of 296

serum samples were taken on alternate days, starting on the 2nd day of the cycle until the 7th day of the next cycle. Altogether, the numbers of serum obtained during menstrual phase I, follicular phase, peri-ovulatory, luteal phase and menstrual phase II were 36, 59, 35, 112 and 54, respectively. All samples were aliquoted and stored at –70°C until assayed. CA 125, CA 15–3, oestradiol and progesterone serum concentrations were measured. Procedures followed were in accordance with the Helsinki declaration on human experimentation of 1975, as revised in 1983, and in accordance with the guidelines for research of our institute.

Assays

The CA 125 II immunoradiometric assay (IRMA) used is a one-step heterologous double determinant test (Centocor Inc., Malvern, PA, USA), employing the M2 murine MAb as capture antibody and the OC 125^{125}I-labelled MAb as tracer antibody for detection of the CA 125 antigen (Kenemans *et al*., 1995).

MUC1 derived PEM was measured with the CA 15–3 radioimmunoassay (Centocor Inc.), a heterologous double determinant assay in which MAb DF3 is employed as tracer and MAb 115D8 as ^{125}I-labelled capture antibody (Tobias *et al*., 1985).

Oestradiol and progesterone concentrations were determined with a double antibody radioimmunoassay (Sorin Biomedica, Saluggia, Italy) and a competitive luminescence immunoassay (Amerlite, Amersham, UK), respectively. The lower detection limits of oestradiol and progesterone were 18 pmol/l and 0.5 nmol/l, respectively.

The detection limits for the CA 125 and CA 15–3 assays are 0.38 kU/l and 0.46 kU/l, respectively. The interassay coefficients of variation for the CA 125 and CA 15–3 assays are 5.7% and 6.5%, respectively. The intra-assay coefficients of variation for the CA 125 and CA 15–3 assays are 2.1% and 7.4%, respectively.

LH was measured using a competitive luminescence immunoassay (Amerlite).

Statistics

Correlations between oestradiol and progesterone serum concentrations with CA 125 and CA 15–3 serum concentrations were calculated using Pearson's correlation coefficient test; P values < 0.05 were considered significant. To enable the comparison of absolute longitudinal marker concentrations among the different subjects, relative marker values were calculated for each serial sample, taking as denominators for CA 125 the maximum measurement occurring during the second menstrual cycle (M2), and for CA 15–3, the maximum measurement in the follicular phase. The Wilcoxon rank sum W test was used to compare CA 125 and CA 15–3 serum concentrations in the different phases of the menstrual cycle.

Results

The median and range CA 125 and CA 15–3 concentrations, as obtained during the different phases of the cycle, are listed in Table I.

In seven out of all 296 sera an elevated CA 125 concentration was found (2.4%, cut-off 35 kU/l). In 16 serum samples an elevated CA 15–3 concentration was observed (5.4%, cut-off 30 kU/l). For CA 125, serum concentrations during menstruation were higher than those measured in other phases of the cycle, but these differences did not reach statistical significance. For CA 15–3, also no statistically different cyclic pattern was found (Table I). This was due to a high inter-individual variation in CA 125 and CA 15–3 serum concentrations. To correct for this variation, relative CA 125 and CA 15–3 serum concentrations were calculated as described earlier. The median and range of relative CA 125 and CA 15–3 serum concentrations are given in Table II. Significantly higher CA 125 serum concentrations were observed during the M1 and M2 ($P < 0.0001$ and $P < 0.001$, respectively), as compared to the follicular phase. During follicular and peri-ovulatory phases, CA 125 serum concentrations were significantly lower as compared to the luteal phase ($P = 0.0024$ and $P = 0.0061$, respectively).

When comparing all phases for each patient separately, mean CA 125 serum concentrations were higher in 19 out of 20 patients in each of the two menstrual phases studied, while in one patient this was only true for the second menstruation. Mean CA 125 serum concentrations during the follicular phase were lower than those found in the luteal phase in 16 out of 20 patients, while during the peri-ovulatory phase mean serum CA 125 concentrations were lower than those in the luteal phase in 15 out of 20 patients (Figure 1a).

For CA 15–3, during the follicular phase, 11 out of 20 patients had higher concentrations, seven patients had lower concentrations and two had concentrations equal as compared to the mean CA 15–3 serum concentrations of the first menstrual phase. Twelve out of 20 patients had higher mean CA 15–3 concentrations during the follicular phase as compared to the second menstrual phase (Figure 1b). However, these differences were not significant. Serum concentrations of CA 15–3 during the menstrual, peri-ovulatory and luteal phases were low and also not statistically different. CA 125 and CA 15–3 serum concentrations in the two patients with tubes that were not occluded did not differ significantly from the concentrations found in patients with occluded tubes (data not shown). A significant negative correlation between CA 125 serum concentrations and oestradiol, and a significant positive correlation between CA 15–3 serum concentrations and oestradiol was observed ($P = 0.033$, $r = –0.1120$ and $P = 0.04$, $r = 0.1487$ respectively) (Figure 2a,b). No significant correlation of CA 125 and CA 15–3 serum concentrations with progesterone serum concentrations was found. Serum concentrations of CA 125, CA 15–3, oestradiol and progesterone in relation to the LH peak are represented in Figure 3a–d.

Discussion

In the present study possible cycle related changes of both serum CA 125 and CA 15–3 in infertile, otherwise healthy women with spontaneous ovulatory cycles were assessed. Until now, no longitudinal studies had been performed in which confounding factors were ruled out, such as endometriosis externa and luteal insufficiency. In this study, laparoscopy excluded occult endometriosis, ovulation was confirmed by LH test and transvaginal ultrasound while luteal insufficiency was excluded on the basis of a biopsy. Also the frequency of blood sampling was high: samples were obtained every second day throughout the whole cycle and during the menstruation of the next cycle.

In all 20 women, CA 125 serum concentrations were highest during menstruation. Earlier reports suggested that this may be due to an easier access of CA 125 from the endometrial

Table I. Median and range absolute CA 125 serum concentrations (kU/l) and absolute CA 15–3 serum concentrations (kU/l) during sequential phases of the menstrual cycle (n = 20). M1 = menstrual phase, F = follicular phase, PO = peri-ovulatory phase, L = luteal phase, M2 = menstrual phase of the next cycle

	M1	F	PO	L	M2	All
CA 125						
median	15.0	11.0	12.0	13.0	13.5	13.0
range	4–43	3–36	3–30	3–42	3–42	3–43
>35 kU/l	5.5%	1.6%	0%	1.8%	3.7%	2.4%
CA 15–3						
median	16.0	17.0	16.0	17.0	17.0	17.0
range	7–35	5–47	7–40	7–37	6–32	5–47
> 30 kU/l	2.7%	6.7%	5.7%	6.3%	3.7%	5.4%

Table II. Median and range of relative CA 125 serum concentrations (kU/l) and relative CA 15–3 serum concentrations (kU/l) throughout the menstrual cycle (n = 20). M1 = menstrual phase, F = follicular phase, PO = peri-ovulatory phase, L = luteal phase, M2 = menstrual phase of the next cycle

	M1[a]	F[b]	PO[c]	L	M2[d]	All
CA 125						
median	0.91	0.76	0.75	0.80	0.93	0.81
maximum value	1.93	0.94	0.95	1.33	1.00	1.93
CA 15–3	M1	F	PO	L	M2	All
median	0.92	0.93	0.91	0.88	0.87	0.88
maximum value	1.15	1.00	1.25	1.25	1.19	1.25

[a]Significantly higher as compared to follicular ($P < 0.0001$), peri-ovulatory ($P < 0.0001$) and luteal phase ($P = 0.0094$).
[b]Significantly lower as compared to luteal phase ($P = 0.0024$).
[c]Significantly lower as compared to luteal phase ($P = 0.0061$).
[d]Significantly higher as compared to follicular ($P < 0.001$), peri-ovulatory ($P < 0.0001$) and luteal phase ($P < 0.0001$).

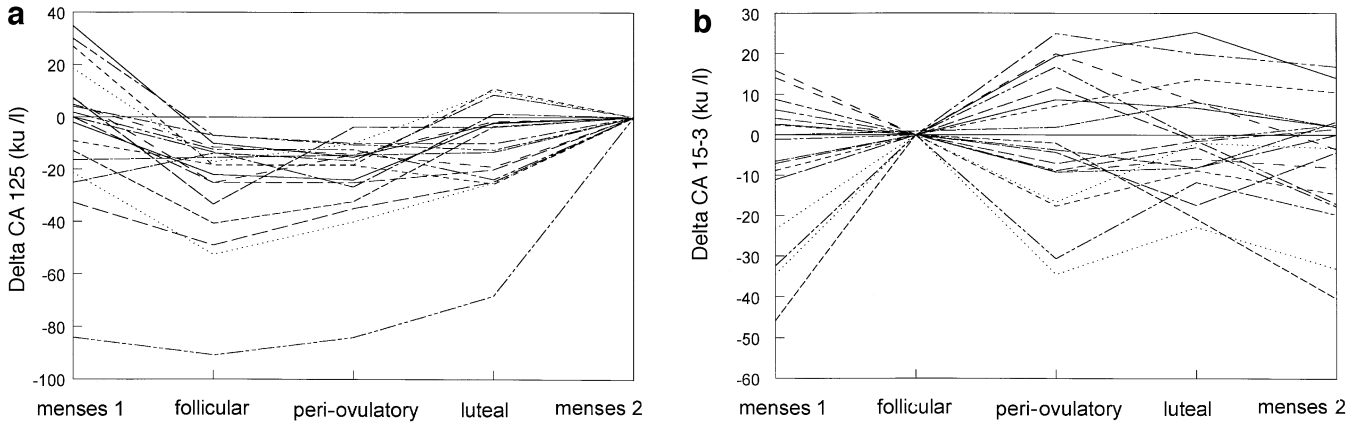

Figure 1. (**a**) Fluctuations of mean CA 125 serum concentrations according to different phases for individual patients with the mean value during the menstrual phase 2 as reference value (n = 20); (**b**) Fluctuations of mean CA 15–3 serum concentrations according to different phases for individual patients with the mean value during the follicular phase as reference value (n = 20).

epithelial lining into the circulation during menstruation (Mastropaolo *et al*., 1986). Other possible explanations for a rise in serum CA 125 during menses are retrograde menstruation and endometriosis externa (Pittaway and Fayez, 1987; Hompes *et al*., 1996). CA 125 could gain access to the abdominal cavity via a tubal reflux, resulting in subsequent absorption via the peritoneal lymphatics or resulting in local inflammatory reactions with subsequent coelomic CA 125 release. In this study, the influence of retrograde menstruation could only be minor because 18 out of 20 patients had occluded tubes. Our observation was confirmed by a study of Abrão *et al*. (1997), in which higher mean CA 125 serum concentrations were observed during menses as compared to the luteal phase in 15 women with a history of bilateral tube ligation.

In this study, lowest CA 125 serum concentrations were observed during the follicular and peri-ovulatory phase and higher CA 125 concentrations during the luteal phase, which is possibly due to a cycle dependent release of CA 125, probably from the endometrium. Interesting in this respect is a study performed by Bischof *et al*. (1986), who observed during the proliferative phase the highest CA 125 concentrations in medium of cultured endometrial stromal cells. In

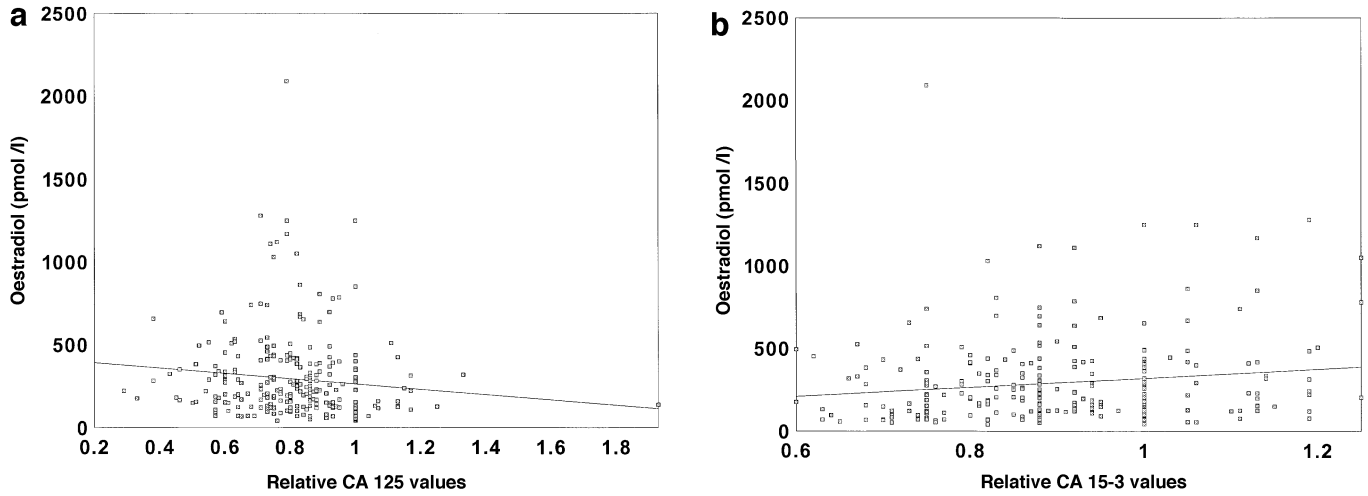

Figure 2. (**a**) Negative correlation between relative CA 125 and oestradiol serum concentrations of all 20 patients ($P < 0.033$); (**b**) Positive correlation between relative CA 15–3 and oestradiol serum concentrations of all 20 patients ($P < 0.007$).

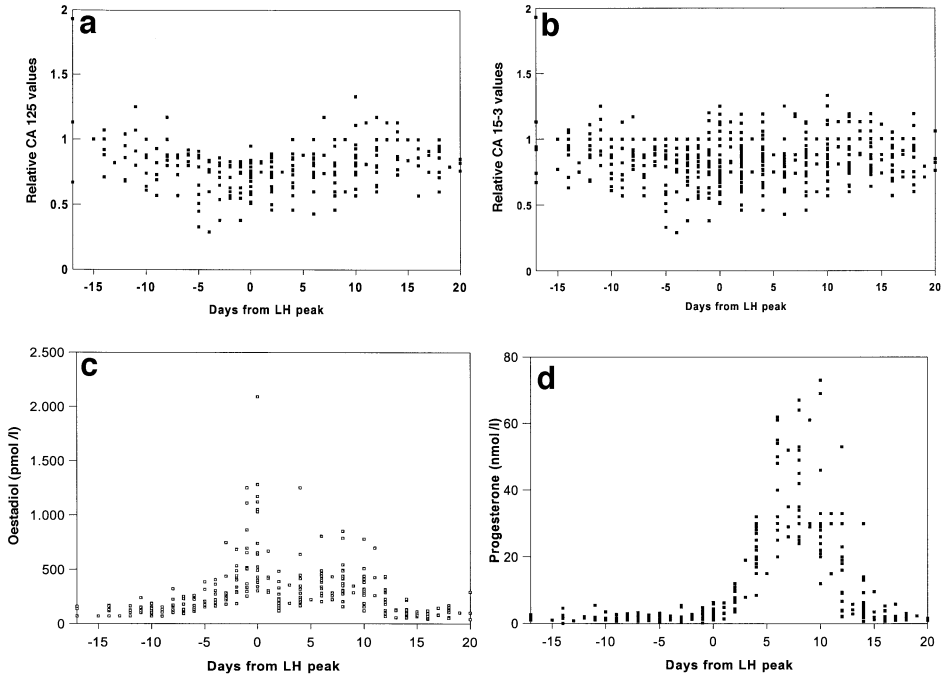

Figure 3a–d. Relative CA 125 and CA 15–3 serum concentrations and absolute oestradiol and progesterone serum concentrations in relation to MUC1 luteinizing hormone (LH) peak.

contrast, others (Weintraub *et al*., 1990; Zeimet *et al*., 1993) reported that the CA 125 serum concentrations were highest during the secretory phase.

This study has revealed a significant negative correlation between oestradiol and CA 125 serum concentrations. The direct effect of ovarian steroids on serum CA 125 content has never been studied, but a hormone dependency has been suggested in studies reporting significantly higher mean CA 125 serum concentrations in premenopausal women as compared to those concentrations obtained in postmenopausal women (Bon *et al*., 1996). Postmenopausal women who had one or both ovaries removed had significantly higher CA 125 values than women who retained both ovaries, independent of whether or not a hysterectomy had been performed (Westhoff *et al*., 1990). Significantly decreased CA 125 concentrations after hysterectomy in both pre- and postmenopausal women have also been reported (Grover *et al*., 1992). In the same study, significantly lower CA 125 concentrations were observed in postmenopausal women receiving hormone replacement therapy suggesting a negative effect of ovarian steroids on serum CA 125 concentrations.

No correlation was found between CA 125 and progesterone serum concentrations. Others reported an inverse relation (Brumsted *et al*., 1990; Lehtovirta *et al*., 1990). Osaza *et al*. (1987) found a positive correlation between serum CA 125 and progesterone concentrations in patients with endometriosis.

Little was known with respect to a possible cycle dependency of MUC1 derived CA 15–3 serum concentrations. In this study, CA 15–3 serum concentrations were low and not significantly different in all phases of the menstrual cycle.

Immunohistologically (Zotter *et al*., 1988) the CA 15–3 antigen is present in endometrial glands. Experiments in mice reported an increase of CA 15–3 during the oestrogenic phase of the cycle, due to a lack of progesterone repression and not because of stimulation by oestrogen (Braga and Gendler, 1993). MUC1 expression in mouse uterine epithelium was shown to be strongly influenced by ovarian steroids, with progesterone down-regulating MUC1 expression (Surveyer *et al*., 1995). *In vivo*, Hey *et al*. (1994) found a low constitutive concentration of MUC1 in the endometrium with up-regulation of MUC1 expression in response to progesterone. The amount of mRNA coding for MUC1 increases about sixfold from the proliferative phase to the early secretory phase. Aplin *et al*. (1994) found low concentrations of human endometrial MUC1 core protein in the proliferative phase of the cycle, and a dramatic increase of MUC1 in cells and luminal gland secretions during the post-ovulatory phase of the cycle, in secretions reaching a maximum after the LH peak on day 6–7. These immunohistochemical findings are not in agreement with the findings of this study that CA 15–3 serum concentrations during the luteal phase are low and statistically not different from all other phases of the cycle. This may be caused by the nature of the CA 15–3 antigen: the glycosylation pattern of the MUC1 molecule in uterine tissue may be different, thus affecting the presentation and/or secretion of the epitopes in serum in such a way that detection of MUC1 by MAb DF3 and 115D8 is altered.

In summary, CA 125 antigen released from the endometrium seems to have access to the lymphatics and the circulation and by way of retrograde menstruation to the peritoneum where local inflammatory reactions of the mesothelium might trigger CA 125 production. The finding in this study of higher CA 125 serum concentrations during the luteal phase correlates with findings showing an increased amount of antigen release by the endometrium in this phase; the mechanism of an enhanced antigen shedding into the circulation remains unclear since the mechanism of retrograde menstruation was ruled out. The increase of CA 125 and not that of CA 15–3 serum concentrations during menstruation may be explained by the different nature of both molecules. Although there is an increasing knowledge about the CA 15–3 molecule and its mechanism of release from the apical cell surface, such information on the CA 125 antigen is still lacking.

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