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CA 125 concentrations in ovarian 'chocolate' cyst fluid can differentiate an endometriotic cyst from a cystic corpus luteum

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In a prospective study, the concentrations of CA 125, 17β-oestradiol and progesterone were assayed in 52 consecutive ovarian cysts, laparoscopically suspected to be endometriomas. Cysts with dark brown 'chocolate' fluid (n = 42) were excised by CO_2-laser endoscopy. Cysts with clear fluid were diagnosed by pathology as follicular cysts (n = 5) or pseudoperitoneal cysts (n = 5). Fluids (n = 53) aspirated during echo-guided puncture for in-vitro fertilization (IVF) were assayed simultaneously. Of the 42 women undergoing a cystectomy, the clinical diagnosis of an endometrioma was confirmed by pathology in only 68%, the other cases being corpora lutea (27%) or follicular cysts (5%). Cyst fluids from corpora lutea had lower CA 125 concentrations (<1000 IU/ml) together with high 17β-oestradiol concentrations (>2000 pg/ml) and/or high progesterone concentrations (>100 ng/ml). Endometriotic cysts had either very high CA 125 concentrations (>10 000 IU/ml) as occurred in 78% or lower CA 125 concentrations (<1000 IU/ml) together with low 17β-oestradiol and/or progesterone concentrations. 'Chocolate' fluid-containing cysts aspirated during IVF had similar concentration profiles of CA 125, 17β-oestradiol and progesterone and the diagnoses derived from these concentrations were not contradicted in 19/27 women undergoing a laparoscopy within 4 months. In eight women, however, with high CA 125 concentrations in their cyst fluid, no endometriotic cysts were found at laparoscopy. Only 68% of cysts containing 'chocolate' material were endometriotic cysts and CA 125 could be useful in making this diagnosis. This method is recommended when dark brown fluid is aspirated in IVF.

Key words: CA 125/corpus luteum/endometrioma/endometriosis/ovarian cyst

Introduction

Cystic ovarian endometriosis is a frequent disorder occurring in 22% of women with infertility and/or pelvic pain (Koninckx *et al.*, 1991a) and is considered to be a cause of infertility. Women suffering this condition are found almost exclusively in the more severe classes III and IV of the revised American Fertility Society (AFS) Classification of endometriosis (The American Fertility Society, 1985). Cystic ovarian endometriosis is associated with pelvic pain and with pelvic adhesions and can be considered an end-point of endometriosis, described recently as a progressive disease (Koninckx *et al.*, 1991a). Since medical treatment only reduces the volume of the endometriotic cysts (Stinegold *et al.*, 1987), surgery is the treatment of choice. Conservative surgical treatment is highly effective, achieving cumulative pregnancy rates of 40% to 60% after both microsurgery and CO_2-laser endoscopic surgery (Gordts *et al.*, 1984; Donnez *et al.*, 1987; Olive and Martin, 1987; Nezhat *et al.*, 1989; Wheeler, 1991).

The diagnosis of cystic ovarian endometriosis cannot be made with certainty by sonography (Sandler and Karo, 1978; Friedman *et al.*, 1985; Boog *et al.*, 1987; Athey and Diment, 1989), and even magnetic resonance imaging (MRI) achieves a sensitivity of only 93% and a specificity of 91% (Scoutt and McCarthy, 1991). The diagnosis is generally made during surgery by the dark brown 'chocolate' appearance of the cyst fluid. This can, however, be misleading, since we repeatedly performed CO_2-laser endoscopic cystectomies for cysts which were later diagnosed by pathology as cystic corpora lutea. In addition, a conclusive diagnosis following the echo-guided puncture of dark brown cyst fluid is generally difficult, if not impossible, although this can be important for the subsequent management of the patient.

CA 125 concentrations in plasma were reported to be elevated in women with advanced endometriosis (Barbieri *et al.*, 1986; Jacobs and Bast, 1989), especially in women with endometriomas and deeply infiltrating endometriosis (Koninckx *et al.*, 1992). We therefore prospectively investigated whether CA 125 concentrations in 'chocolate' cyst fluid could be used to diagnose ovarian 'chocolate' cysts as endometriomas or corpora lutea.

Materials and methods

Patients and cyst fluid

In women undergoing CO_2-laser endoscopic surgery for infertility and/or pelvic pain, ovarian cysts which were clinically suspected to be cystic ovarian endometriomas were aspirated (n = 52). If dark brown or 'chocolate' fluid was aspirated (n = 42), the cysts were excised with a CO_2-laser (Sharplan 1060, Laser Industries Ltd, Tel Aviv, Israel) and the remaining ovarian tissue closed with fibrin sealant (Tissucol, N.V. Immuno, Brussels, Belgium). If clear fluid was aspirated (n = 10), and

a follicular cyst or a pseudoperitoneal cyst was suspected, a biopsy only was taken from the cyst wall. When the slightest suspicion of malignancy existed, a biopsy was sent for frozen sectioning.

The pathological criterion used for an endometriotic cyst was the identification of endometrial tissue, including both glandular and stromal elements. A follicular cyst showed granulosa and theca cell layers, separated by a basal lamina. The typical thick convoluted lining of a corpus luteum was absent. A cyst derived from the corpus luteum showed evidence of prior ovulation. The cyst lining was convoluted and lined by a layer of luteinized granulosa cells, surrounded by luteinized theca cells. A 'pseudoperitoneal' cyst was lined by attenuated mesothelial cells, resting upon a fibrous stroma that often contained chronic inflammatory cells.

Also chocolate cyst fluids (n = 53), aspirated during vaginal ultrasound-guided oocyte retrieval in women undergoing in-vitro fertilization (IVF), were included in this study. These women had had ovarian stimulation with human menopausal gonadotrophins. In 27 women, a laparoscopy was performed subsequently within 4 months.

Assays

CA 125 concentrations were assayed using the Centocor radioimmunoassay kit as described for peritoneal fluid (Koninckx *et al.*, 1992). Briefly, cyst fluids were diluted at least four times in male plasma. For the CA 125 assay the fluids were serially diluted until two or more consecutive dilutions yielded similar results. This was necessary to eliminate the 'hook' effect, which underestimated the concentrations in undiluted samples. The concentrations of 17β-oestradiol and progesterone were assayed by raidoimmunoassay after cyclohexane–ethylacetate (1:1) extraction, as described by Koninckx *et al.* (1980).

Statistics

Statistical calculations were performed with the SAS package (SAS User's Guide, 1985) using Spearman correlations

Results

Macroscopically, cystic ovarian endometriosis could not be differentiated from cystic corpora lutea. Out of 42 cystectomies performed because of clinically diagnosed cystic ovarian endometriosis, 11 (27%) turned out to be corpora lutea, two (5%) follicular cysts and only 29 (68%) endometriomas. Also, the macroscopic appearance of the cyst fluid could not be used to diagnose cystic ovarian endometriosis. 'Chocolate' fluid was found in 28 endometriomas and two corpora lutea. Dark brown fluid was found in one endometrioma, seven corpora lutea and two follicular cysts, whereas red 'blood' fluid was found in two corpora lutea.

In ovarian endometriotic cysts CA 125 concentrations were very high ranging from 10^3 IU/ml to $>10^6$ IU/ml. In the majority of corpora lutea the concentrations of progesterone and of 17β-oestradiol were noticeably elevated (Figure 1). Follicular cysts contained high concentrations of 17β-oestradiol and progesterone and only slightly elevated CA 125 concentrations. Pseudoperitoneal cysts showed 17β-oestradiol and progesterone

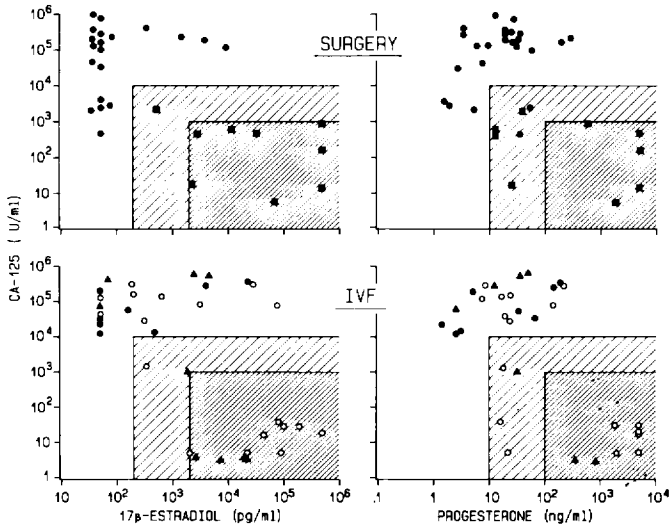

Fig. 1. Concentrations of CA 125 and 17β-oestradiol or progesterone in 'chocolate' fluid from ovarian cysts excised during surgery (●, endometriotic cyst; ■, corpus luteum) in spontaneous cycles or aspirated during echo-guided puncture for in-vitro fertilization (IVF) (●, endometriotic cysts; ▲, no subsequent surgery; ○, no endometriotic cyst during subsequent surgery). The diagnostic areas for endometriotic cysts (white), corpora lutea (▩), and the grey zone (▨) are indicated.

concentrations which were comparable with serum concentrations, and slightly elevated concentrations of CA 125.

The CA 125 together with the 17β-oestradiol and progesterone concentrations in 'chocolate' fluid could differentiate cystic ovarian endometriosis from haemorrhagic corpora lutea at least in women with spontaneous cycles (Figure 1). When the cyst fluid CA 125 concentration was $>10^4$ IU/ml (78% of all endometriomas), an endometriotic cyst could be diagnosed with a sensitivity and specificity of 100%. When the cyst fluid CA 125 concentration was <1000 IU/ml and the 17β-oestradiol concentration >2000 pg/ml or the progesterone concentration >100 ng/ml a cystic corpus lutem could be diagnosed with a sensitivity and a specificity of 100%. If only progesterone or 17β-oestradiol were used, together with CA 125, a grey zone existed. When the CA 125 concentration was between 1000 and 10 000 IU/ml and the 17β-oestradiol concentration >200 pg/ml or the progesterone concentration >10 ng/ml, or when the CA 125 concentration was <1000 U/ml and the 17β-oestradiol concentration was between 200 and 2000 pg/ml or the progesterone concentration between 10 and 100 ng/ml, both corpora lutea and endometriotic cysts could be found. The combined use, however, of CA 125, 17β-oestradiol and progesterone allowed a correct diagnosis in all cases.

In endometriomas, the CA 125 concentration was negatively correlated with the volume of the cyst $R = -0.36$, $P = 0.05$, $n = 28$). Also in corpora lutea, the CA 125 concentration was correlated negatively with the volume of the cyst ($R = -0.73$, $P = 0.03$, $n = 8$). Moreover the 17β-oestradiol and progesterone concentrations were interrelated ($R = 0.78$, $P = 0.05$, $n = 8$).

The concentration profiles and concentration ranges in the dark brown and/or chocolate cystic fluid aspirated during IVF were

Table I. Concentrations of CA-125, 17β-oestradiol and progesterone in endometriomas, (old) corpora lutea, follicular cysts and pseudoperitoneal cysts during surgery. The means (25–75th percentiles) are given

	n	CA-125 (U/ml)	17β-oestradiol (pg/ml)	Progesterone (ng/ml)
Endometrioma	28	227 695 (39 097–310 220)	624 (50–50)*	36 (5–32)
Corpus luteum (haemorrhagic)	8	431 (82–760)	202 426 (7 450–500 000)	2182 (25–5 000)
Follicular cyst	5	77 (70–155)	133 520 (5 040–262 000)	347 (50–645)
Blood	3	20 (16–29)	78 493 (1 480–190 000)	3341 (25–5000)
Pseudoperitoneal cyst)	5	6 102 (25–7 859)	625 (50–1200)	20 (8–28)

Data are of mean (25–75th percentile).
*Highly skewed distribution.

comparable with those found during surgery (Figure 1). The diagnosis made, based upon the concentrations of CA 125, progesterone and 17β-oestradiol, was never proven wrong in any of the 27 women undergoing a laparoscopy within 4 months. When during subsequent surgery an endometriotic cyst was found ($n = 8$), all cyst fluids which had been aspirated during IVF had CA 125 concentrations >10 000 IU/ml. An endometriotic cyst was not found in any of the women with low concentrations of CA 125 and high concentrations of 17β-oestradiol or progesterone in their cyst fluid. However, in eight out of the 27 women, with high concentrations of CA 125 in their chocolate cyst fluid, no endometriotic cysts were found during subsequent surgery.

Discussion

The elevated plasma concentrations of CA 125 in women with advanced endometriosis (Barbieri *et al.*, 1986; Jacobs and Bast, 1989) were recently shown to be caused mainly by ovarian endometriotic cysts and deeply infiltrating endometriosis (Koninckx *et al.*, 1992). Moreover, the epithelial lining of ovarian endometriotic cysts was shown to express CA 125 (Barbieri *et al.*, 1986; F.Cornillie, unpublished observation) whereas the high molecular weight of CA 125 (Davis *et al.*, 1986) will impair diffusion. Thus it is not surprising that CA 125 concentrations in the 'chocolate' cyst fluid of endometriomas are considerably elevated, as reported by Pittaway *et al.* (1987).

The differentiation of ovarian endometriotic cysts from cystic corpora lutea is clinically important, since cystectomies for cystic corpora lutea should be avoided, especially in women with infertility because of the risk of post-operative adhesion formation. We were surprised to find that our laparoscopic diagnosis which takes into account the macroscopical appearance of the ovary, the 'chocolate' cyst fluid and the laparoscopic appearance of the cyst wall, was so frequently (32%) wrong during this prospective study. We therefore suggest that during infertility surgery, the diagnosis should be made by pathology on frozen sections, as is done when malignancy is suspected. A closer inspection of the cyst wall by cystoscopy (Brosens and Puttemans, 1989) could be used, but the diagnostic accuracy of this method remains to be determined. Also CA 125 and steroid concentrations in cyst fluid could be used if a rapid method of measurement or estimation such as a dip-stick becomes available.

When during an echo-guided puncture, e.g. for IVF 'chocolate' fluid is aspirated, the diagnosis of a cystic corpus luteum or an endometriotic cyst is even more difficult than during surgery. Imaging can help to make a diagnosis, but cannot yet differentiate with certainty between a cystic corpus luteum and an endometriotic cyst. Magnetic resonance imaging has a sensitivity and specificity of 93% and 91% respectively, whereas the diagnostic accuracy of ultrasound (Sandler and Karo, 1978; Friedman *et al.*, 1985; Boog *et al.*, 1987; Athey and Diment, 1989) is even lower. Our data suggest that the concentrations of CA 125, 17β-oestradiol and progesterone could be used to make the conclusive diagnosis. In women with a natural cycle, a correct diagnosis could be made with 100% sensitivity and specificity and the same diagnostic criteria can probably be used in women undergoing ovulation induction e.g. for IVF. Indeed, the similarity of the concentration profiles and ranges found in 'chocolate' fluid during natural cycles and during ovulation induction suggests that both situations have comparable concentration ranges. The fact that plasma concentrations of CA 125 are known to be slightly elevated during ovulation induction (Jäger *et al.*, 1987) probably does not affect the cyst fluid concentrations significantly, since this increase is small in comparison with the very high concentrations found in endometriotic cysts. The strongest points of this study, bearing in mind the restrictions imposed by the small series, are the findings in the 27 women undergoing a laparoscopy within 4 months following the echo-guided aspiration of 'chocolate' fluid, indicating that these diagnostic criteria were useful. Indeed, no endometriotic cysts were found during subsequent surgery in women with low CA 125 and high 17β-oestradiol and/or progesterone concentrations in their cyst fluid during puncture. Moreover, in all women in whom an endometriotic cyst was found at laparoscopy, this had been predicted by CA 125 and steroid concentrations during earlier puncture. A troublesome but probably important observation, however, was that in eight women with high CA 125 concentrations in their 'chocolate' fluid, no endometriotic cysts were found during subsequent surgery. We speculate that some endometriotic cysts did not refill within 4 months after complete aspiration and thus were missed during laparoscopy. This interpretation is consistent with our previous observations that in some women, endometriomas do not reform rapidly following aspiration, rinsing and 3 months' treatment with a luteinizing hormone-releasing hormone agonist (Koninckx *et al.*, 1991b).

CA 125 is a tumour-associated antigen expressed in derivatives of coelomic epithelium, and is mostly used as a tumour marker for epithelial ovarian cancer. Although CA 125 could not be

detected in granulosa or theca cells, the concentrations in (persistent) follicular cysts were definitely increased, suggesting a local secretion. Also, in most pseudoperitoneal cysts, the concentrations of CA 125 were markedly elevated. At this time we can only speculate that local peritoneal irritation, or possibly minimal endometriotic lesions within these cysts, could cause this elevation.

The negative correlation between cyst volume and CA 125 concentrations was unexpected but could possibly be explained as follows. CA 125 is secreted by endometriotic epithelial cells lining the cyst wall. Since larger cysts have a smaller surface area/volume ratio than smaller cysts, this could explain that CA 125 concentrations are lower in larger cysts.

In conclusion, cysts containing dark brown 'chocolate' fluid are corpora lutea or endometriotic cysts in 27% and 68% of cases respectively. A correct diagnosis can be made using the concentrations of CA 125 and 17β-oestradiol and/or progesterone in the cyst fluid in natural cycles, and evidence is presented to suggest that the same criteria can be used during ovulation induction. During surgery this method is not yet clinically useful, unless a rapid assay method, e.g. dip-stick, becomes available. For aspirates obtained during echo-guided puncture, such as in IVF, this method could become an important diagnostic tool. Finally it is suggested that after a complete aspiration, some endometriotic cysts do not refill rapidly, i.e. not within 4 months, in some 50% of women. This may be physiologically and clinically important.

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