SHORT COMMUNICATION

Peritoneal trauma releases CA125?

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CA125 is a high molecular weight glycoprotein that is detected in tissues derived from foetal embryonic coelomic epithelium (Kabawat et al., 1983). Serum CA125 levels are elevated in 80% of patients with epithelial ovarian cancer (Bast et al., 1983) although up to 70% of patients with small volume disease will have false negative values (Schilthuis et al., 1987; Niloff et al., 1985; Atack et al., 1986). Serum levels may in part depend on a tumour-peritoneal cavity-blood concentration gradient (Bast et al., 1981; Bergmann et al., 1987; Fleuren et al., 1987) and as ovarian cancer is a disease predominantly confined to the peritoneal cavity, peritoneal washings may be a more sensitive marker of small volume disease (Allegra et al., 1986). This has suggested the possibility that peritoneal lavage fluid (PLF) CA125 may be a useful staging tool at laparoscopy, and possibly at laparotomy in the detection of sub-clinical disease.

As a preliminary investigation in the evaluation of peritoneal lavage fluid (PLF) CA125 as a marker of minimal residual disease in ovarian cancer, we wished to measure CA125 levels in the peritoneal lavage fluid obtained from healthy controls. Since there are isolated reports of serum CA125 levels rising as a consequence of abdominal surgery (Krebs et al., 1986, Cruickshank et al., 1987), it was essential to assess the effect of surgery on PLF CA125 levels.

We performed the study in two groups of patients. In group I, pre-operative serum and peri-operative peritoneal lavage fluid were obtained from healthy pre-menopausal women undergoing either hysterectomy for dysfunctional uterine bleeding (n=15) or laparoscopy (n=40). The indications for laparoscopy were sterilization (n=28), unexplained pelvic pain (n=5), or infertility (n=7). No evidence of disease, in particular endometriosis, was found at operation although there was histological evidence of adenomyosis in three of the hysterectomy specimens.

Peritoneal lavage was performed at laparoscopy after the introduction of the laparoscope, whilst in patients undergoing hysterectomy, it was performed immediately after opening the peritoneal cavity, great care being taken to avoid contamination with blood. Peritoneal lavage was performed with 11 0.9% saline that was left in situ for 5 min before a 20 ml sample was taken and added to a plastic universal container with 1 ml 3% sodium citrate. The operating table was repeatedly tilted to ensure as uniform a distribution as possible.

Group II comprised 6 further patients undergoing hysterectomy for dysfunctional bleeding (median age 36; range 31–42). In this group, the anterior abdominal wall was opened normally down to the peritoneum. Peritoneal lavage was then performed, instilling 11 0.9% saline via a small peritoneal incision just sufficient for a 12 g urinary catheter to pass through. A sample of PLF was obtained after a dwell time of 5 min. The peritoneum was then opened normally and a second sample of fluid taken 5 min later. Peritoneal biopsies were obtained for immunohistological studies. Serum CA125 levels were measured on each of the first 5 post-operative days.

The blood and the PLF were centrifuged within 4 h. The serum and PLF supernatant were stored at -20°C until assayed using a simultaneous sandwich IRMA (CIS, UK). All measurements were performed in duplicate. PLF total protein concentration was measured using a manual Pronase S dye binding method with colour measurement on a Kontron UV spectrophotometer.

Immunohistochemical detection of CA125 in the peritoneal biopsies was performed on snap-frozen material. OC125 murine monoclonal antibody was purchased in kit form (CIS, UK) and an avidin-biotin immunoperoxidase technique was used in accordance with the manufacturer’s instructions.

Natural logarithmic transformation of serum CA125, PLF CA125 and protein values was employed to normalise their positively skewed distributions. Student’s t-test was used to determine statistical significance of the differences between means. In group I, a joint regression analysis of PLF CA125 on patients’ age was performed (Mather, 1964). In group II the post-operative serum CA125 values for each patient were analysed as a percentage change from the baseline pre-operative values and for each time point means and 95% confidence limits were computed. The analysis was performed using a VAX 11/730 minicomputer at the West Midlands CRC Clinical Trials Unit using programs from the BMDP statistical software package (Dixon et al., 1985).

Immunohistochemical studies demonstrated CA125 positivity in the mesothelial cells lining the peritoneum in all the specimens.

In group I, the CA125 concentration was significantly higher in PLF obtained at laparotomy than that obtained at laparoscopy whilst there was no significant difference between the serum CA125 levels or the PLF protein concentration (Table I).

Patients undergoing hysterectomy were significantly older than the laparoscopy group. However, in a joint regression analysis, there was no heterogeneity of regression between the two groups (F1,51 = 0.4; P > 0.2) and there was no evidence for an association between PLF CA125 and age (F1,50 = 0.2; P > 0.20). There was no significant correlation between PLF protein concentration and PLF CA125 (r = 0.057; P = 0.8) or between serum and PLF CA125 levels (r = 0.23; P = 0.092).

In group II, PLF CA125 levels after the peritoneum had been widely opened were significantly higher than when the peritoneal lavage had been performed via a small peritoneal incision (Table II). Despite this rise, there is no post-operative elevation in serum CA125, although the numbers involved are small (Figure 1).

Using PLF CA125 values obtained at laparoscopy, a cut-off point of 90 U ml⁻¹ was determined, which would be exceeded by only 1% of the normal population (i.e., mean

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indeed in (1983) and the breached into the data). Serum cut-off and the involvement, and releases CA125 levels in patients undergoing data PLF that indicate a higher protein serum CA125 in CA125 is also sensitive for peritoneal lavage. This is likely to be due to the contamination of PLF with blood or tissue fluids containing CA125 as the rise in CA125 is independent of the PLF protein concentration.

Immunohistology confirms the findings of Kabawat et al. (1983) that CA125 is expressed in normal adult peritoneum, and may account for the elevation of serum CA125 observed in a number of conditions that involve the peritoneum (Malkasian et al., 1986; Barbieri et al., 1986; Halila et al., 1986). Surgical trauma may therefore release CA125 and indeed Cruickshank et al. (1987) noted serum CA125 elevation in five patients with ovarian cancer between the third and the 16th post-operative day, though this may not have been statistically significant. It is also apparent that in ovarian and other cancers the extent of peritoneal involvement strongly influences serum levels (Duk et al., 1986; Schihthuis et al., 1987; Fleuren et al., 1987). The higher serum levels observed in conjunction with peritoneal metastases may be because the peritoneal basement membrane is breached by tumour, enabling the tumour antigens to enter into the peripheral circulation (Fleuren et al., 1987). However it is also possible that the peritoneum itself produces and releases CA125 antigen as a reaction to metastatic involvement, as non-malignant conditions that either inflame or involve the peritoneum are associated with elevated serum levels (Barbieri et al., 1986; Halila et al., 1986). Our findings provide further evidence that non-malignant peritoneal events release CA125 and illustrate further the nonspecificity of CA125 as a marker of ovarian cancer. In our study, despite the elevation of PLF CA125 as a result of peritoneal trauma there was no significant rise in the post-operative serum levels but the numbers are small and further study is warranted.

We hope to evaluate peritoneal lavage as a diagnostic procedure in ovarian cancer patients both at laparoscopy and in an out-patient context for the monitoring and detection of small volume residual or progressive disease. As a result of this preliminary study we conclude that control PLF CA125 data must be collected at laparoscopy, not at laparotomy. If a similar cut-off point is adopted as for serum CA125 (i.e., to produce a false positive rate of 1% in healthy controls; Bast et al., 1983) the upper limit of the normal range is 90 U ml⁻¹. Discriminant analysis using control and cancer patients' data might give a more useful cut-off point, especially as PLF CA125 is most likely to be used in monitoring ovarian cancer and not as a population screening tool. In addition, serum and PLF CA125 used in conjunction with other diagnostic tests could be combined using a discriminant analysis to produce an index that would be more useful for predicting the presence of disease than PLF alone.

Table I Comparison between laparoscopy and laparotomy patients in group 1

|         | Laparoscopy [n=40] | Laparotomy [n=15] |
|---------|-------------------|-------------------|
|         | Mean CI           | Mean CI           |
| Age     | 32 30-34          | 41 39-43          |
| Serum CA125* | 18 15-22       | 21 16-27          |
| PLF CA125* | 25 21-30        | 158 137-183       |
| PLF protein* | 0.36 0.23-0.55  | 0.58 0.26-1.13    |

*Tests of significance carried out after log transformation; Separate variance t-test; Pooled variance t-test.

Table II PLF and post-operative serum CA125 results in group II patients

| Patient | PLF CA125 | Post-operative serum CA125 |
|---------|-----------|---------------------------|
|         | Small incision | Large incision | 0 | 1 | 2 | 3 | 4 | 5 |
| 1       | 47         | 68            | 21 | 19 | 22 | 26 | 24 | 23 |
| 2       | 26         | 106           | 54 | 61 | 38 | 80 | 80 | 68 |
| 3       | 41         | 94            | 53 | 41 | 37 | 34 | 33 | 35 |
| 4       | 60         | 277           | 24 | 30 | 34 | 56 | 49 | 50 |
| 5       | 21         | 46            | 20 | 18 | 20 | 24 | 22 | 26 |
| 6       | 36         | 137           | 24 | 18 | 20 | 22 | 22 | 16 |

Mean [95% CI] 39 [24-54] 121 [34-208]

*Paired tₜ = 2.7, P = 0.038.

Figure 1 Percentage changes of pre-operative serum CA125 following laparotomy (mean and 95% confidence intervals).
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