Soluble adhesion molecules in serum and cyst fluid from patients with cystic tumours of the ovary

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The potential of the soluble forms of the adhesion molecules ICAM-1 (sICAM-1), CD44std (sCD44std) and E-cadherin (sE-cadherin) was tested for the diagnosis of benign and malignant cystic epithelial tumours of the ovary. Concentrations of sICAM-1, sCD44 std and sE-cadherin were measured by enzyme-linked immunosorbent assay (ELISA) in the serum and cyst fluid obtained from 23 patients with luteal cysts, 29 with cystadenomas, nine with dermoid cysts, five with borderline tumours and 11 with carcinomas. Serum concentrations of sICAM-1, but not of sCD44std and sE-cadherin, were constantly elevated compared with normal controls. Cyst fluid concentrations of sICAM-1, sCD44std and sE-cadherin were elevated in borderline and malignant tumours compared with cystadenomas (P = 0.034, 0.006 and 0.001, respectively). In conclusion, our results suggest that serum concentrations of adhesion molecules have no diagnostic value in ovarian tumours, whereas cyst fluid concentrations may facilitate distinction between benign lesions and borderline or malignant tumours.

Key words: adhesion molecules/cadherins/CD44/ICAM-1/ovarian tumours

Introduction

Adhesion molecules play a pivotal role in carcinogenesis. Abnormal expression of structural adhesion molecules such as cadherins, integrins and CD44 proteins by neoplastic cells is likely to be an important determinant of local invasion and metastatic dissemination (Matsumura and Tarin, 1992; Albelda, 1993). In addition, increased expression of cytokine-dependent adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), contributes to the regulation of the interactions between neoplastic cells and the immune cells of the host (Johnson, 1991).

Most cell adhesion molecules exist in two forms: a membrane-inserted form, detectable on the cell surface; and a soluble form, detectable in the serum and in biological fluids. The soluble forms of several adhesion molecules, including ICAM-1 (sICAM-1), E-cadherin (sE-cadherin) and CD44 proteins (sCD44), have recently emerged as novel and potentially useful tumour markers (Seth et al., 1991; Tarin and Matsumura, 1994). The adverse prognostic value of elevated circulating concentrations of sICAM-1, sE-cadherin and sCD44 isoforms has been underlined in several types of neoplasia, including adenocarcinomas of various origins, tumours of the central nervous system and haematological malignancies (Banks et al., 1993; Hyodo et al., 1993; Gao et al., 1994; Ristamaki et al., 1994). In the same way, elevated concentrations of soluble adhesion molecules in biological fluids in contact with neoplastic cells have proven to be of diagnostic and prognostic value in certain cancers, such as those of the bladder (Jackson et al., 1993) and stomach (Matsumura et al., 1992).

Little is known about the possible clinical relevance of the soluble forms of cell adhesion molecules in the management of ovarian epithelial tumours (Giavazzi et al., 1994; Sliutz et al., 1995). A large array of tumour markers has been proposed for the diagnosis of ovarian disease, but most of these markers—including the widely used CA125—lack sensitivity and/or specificity, particularly for the distinction between benign and malignant lesions (Montag, 1990; Menczer et al., 1993; Chapron et al., 1996). It is therefore important to search for new markers which may possibly improve the early diagnosis, the assessment of disease status and the evaluation of metastatic potential and prognosis in ovarian cancer (Trope, 1981). Recent studies have shown that, as in other epithelial tumours, alterations in the expression of cell adhesion molecules, including cadherins (Inoue et al., 1992; Risinger et al., 1994; Darai et al., 1997) and CD44 proteins (Cannistra et al., 1995; Sliutz et al., 1995; Uhl-Steidl et al., 1995; Darai et al., 1998), are frequent in both borderline and malignant tumours of the ovary. We were therefore prompted to test whether the determination of the concentrations of soluble forms of ICAM-1, E-cadherin and CD44 in either serum or cyst fluid may be of interest for the diagnosis of cystic epithelial ovarian tumours.

Materials and methods

Study design

All patients seen between September 1995 and April 1996 in the Service de Gynécologie-Obstétrique, Hôpital Bichat-Claude Bernard, Paris and presenting with a cystic ovarian mass were eligible for the study. All patients included in the study group had given their informed consent. Serum samples were obtained immediately before the beginning of the surgical procedure. Cyst fluid samples were
duplicate and the mean value was taken for the analysis.

determined by use of a standard curve. All assays were performed in

450 and 492 nm, respectively. Concentrations of immunoreactive

onation with tetramethylbenzidine (for sICAM-1 and sCD44std) or
conjugated primary antibody solution provided. After substrate incuba-

serum samples were incubated for 90 min with the peroxidase-

Vienna, Austria) and sE-cadherin (Takara, Kyoto, Japan) in serum

Commercially available enzyme-linked immunosorbent assay

(ELISA) kits were used for the determination of sICAM-1 (R&D

Cells (for sE-cadherin), the enzyme reaction was

No result could be obtained in fluid samples obtained from most
dermoid cysts. For the other types of ovarian cysts, aliquots of 25 µl
of cyst fluid were sufficient to provide reliable results. The intra-
assay coefficient of variation for samples of cyst fluid assayed in
replicates of 10 was verified as 4.1% to 6.5%, and the inter-assay
coefficient of variation for samples of cyst fluid assayed in duplicate
by two operators as 8% to 14.5%. These figures compared well with
those provided by the manufacturers for serum samples.

Normal serum values (mean ± SD) determined previously in our
laboratory for 20 healthy subjects were 208 ± 55 ng/ml for sICAM-1,
470 ± 110 ng/ml for sCD44std and 2900 ± 510 ng/ml for sE-cadherin.

Statistical analysis

For statistical evaluation, Fisher’s exact test, Wilcoxon two-sample
test and Spearman-rank correlation test were used. A P value < 0.05
was considered significant.

Results

Serum concentrations of sICAM-1, sCD44std and sE-
cadherin

Serum concentrations of sICAM-1, sCD44std and E-cadherin
in patients with luteal cysts, dermoid tumours, cystadenomas
and malignant tumours of the ovary are listed in Table II. There
was no statistically significant difference between the mean serum concentrations of either sICAM-1, sCD44std or
E-cadherin in the four groups of ovarian lesions.

Cyst fluid concentrations of sICAM-1

Cyst fluid concentrations of sICAM-1 in luteal cysts, dermoid
tumours, cystadenomas and malignant tumours of the ovary
are listed in Table II. The concentration of sICAM-1 in cyst
fluid was significantly higher in borderline and malignant
tumours than in benign cystadenomas (P = 0.034). Moreover,
cyst fluid concentrations of sICAM-1 were also significantly
higher in borderline and malignant tumours than in luteal cysts
(P = 0.011). In benign cystadenomas, there was no apparent
difference between serous and mucinous lesions (249 ± 247

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<thead>
<tr>
<th>Table I. Patient characteristics</th>
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<th>Cystadenomas (n = 29)</th>
<th>Dermoid tumours (n = 9)</th>
<th>Borderline tumours (n = 5)</th>
<th>Malignant tumours (n = 11)</th>
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<tr>
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<td>34.5</td>
<td>39.3</td>
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<td>0</td>
<td>3</td>
</tr>
<tr>
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<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
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<td>Stage IV</td>
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FIGO = International Federation of Gynecology and Obstetrics.

obtained by cyst puncture. For macroscopically benign lesions, cyst
puncture was performed prior to surgical resection. For lesions
macroscopically suspicious for malignancy, cyst puncture was done
after extraction of the tumour by laparotomy.

Histological typing was performed according to the recommenda-
tions of the International Federation of Gynecology and Obstetrics
(1987). For the diagnosis of borderline tumours, histological criteria
used in the study included: (i) stratification of the epithelial lining of
the papillae with formation of microscopic papillary projections or
tufts arising from the epithelial lining of the papillae; (ii) nuclear
atypia; (iii) mitotic activity; (iv) intracystic clusters of free-floating
cells; and (v) absence of stromal invasion. In the case of mucinous
tumours, the presence of foci of epithelial cell stratification greater
than three layers was considered diagnostic of carcinoma, according
to the criteria of Hart and Norris (1973).

Patients

The study group consisted of 77 patients. The final clinical and
histological diagnoses were: (i) luteal cysts in 23 patients (unilateral
in 22, bilateral in one); (ii) dermoid cysts in nine patients; (iii)
cystadenoma in 29 patients (serous in 22 cases, mucinous in seven);
(iv) borderline tumours in five patients and overt malignant tumours
in 11 patients. Patient characteristics are summarized in Table I.

Assay for soluble adhesion molecules in serum and cyst fluid samples

Commercially available enzyme-linked immunosorbent assay
(ELISA) kits were used for the determination of sICAM-1 (R&D
Systems, Abingdon, UK), sCD44 standard (sCD44std) (BenderMed,
Vienna, Austria) and sE-cadherin (Takara, Kyoto, Japan) in serum
and cyst fluid samples. The technique was performed according to
the manufacturers’ instructions. Briefly, duplicate test standards and
serum samples were incubated for 90 min with the peroxidase-
conjugated primary antibody solution provided. After substrate incuba-
tion with tetramethylbenzidine (for sICAM-1 and sCD44std) or
o-phenylenediamine (for sE-cadherin), the enzyme reaction was
stopped and the optical density was measured photometrically at
450 and 492 nm, respectively. Concentrations of immunoreactive
sICAM-1, sCD44std and sE-cadherin (expressed in ng/ml) were
determined by use of a standard curve. All assays were performed in
duplicate and the mean value was taken for the analysis.
Table II. Serum and cyst fluid concentrations (ng/ml) of sICAM-1, sCD44std and sE-cadherin in 77 patients with cystic lesions of the ovary. Values are median (range).

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Cyst fluid</th>
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<tr>
<td>sICAM-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteal cysts</td>
<td>452 (40–990)</td>
<td>555 (40–1000)</td>
</tr>
<tr>
<td>Dermoid tumours</td>
<td>520 (500–800)</td>
<td>ND</td>
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<tr>
<td>Cystadenomas</td>
<td>660 (210–1000)</td>
<td>550 (40–1100)</td>
</tr>
<tr>
<td>Borderline/malignant tumours</td>
<td>320 (100–980)</td>
<td>1030 (190–2100)</td>
</tr>
<tr>
<td>sCD44std</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteal cysts</td>
<td>517 (240–1200)</td>
<td>401 (175–960)</td>
</tr>
<tr>
<td>Dermoid tumours</td>
<td>545 (285–1200)</td>
<td>ND</td>
</tr>
<tr>
<td>Cystadenomas</td>
<td>330 (40–1050)</td>
<td>135 (37–900)</td>
</tr>
<tr>
<td>Borderline/malignant tumours</td>
<td>510 (160–1200)</td>
<td>337 (300–375)</td>
</tr>
<tr>
<td>sE-cadherin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteal cysts</td>
<td>3677 (1150–9500)</td>
<td>2035 (200–9750)</td>
</tr>
<tr>
<td>Dermoid tumours</td>
<td>2325 (1150–8000)</td>
<td>ND</td>
</tr>
<tr>
<td>Cystadenomas</td>
<td>2200 (400–10 000)</td>
<td>2000 (20–8750)</td>
</tr>
<tr>
<td>Borderline/malignant tumours</td>
<td>2250 (900–13 000)</td>
<td>14 500 (2200–27 000)</td>
</tr>
</tbody>
</table>

*p < 0.034, 

bp = 0.011, 

*c = 0.006, 

*dp < 0.001.

and 229 ± 282 ng/ml, respectively). The number of cases of mucinous lesions included in the group of borderline and malignant tumours was too low to allow any statistical evaluation.

Cyst fluid concentrations of sCD44std

Cyst fluid concentrations of sCD44std in luteal cysts, dermoid tumours, cystadenomas and malignant tumours of the ovary are listed in Table II. Mean cyst fluid concentrations of sCD44std were significantly higher in malignant tumours than in cystadenomas (P = 0.006), but were not statistically different between malignant tumours and luteal cysts (P = 0.332). Among benign cystadenomas, there was no apparent difference between serous and mucinous lesions (524 ± 347 and 577 ± 346 ng/ml, respectively).

Cyst fluid concentrations of sE-cadherin

Concentrations of sE-cadherin in luteal cysts, dermoid tumours, cystadenomas and malignant tumours of the ovary are listed in Table II. Mean cyst fluid concentrations of sE-cadherin were significantly higher in borderline and malignant tumours than in cystadenomas (P < 0.001) and were also significantly higher in borderline and malignant tumours than in luteal cysts (P < 0.001). Among benign cystadenomas, there was no apparent difference between serous and mucinous lesions (2505 ± 2214 and 3214 ± 2810 ng/ml, respectively).

Comparative distribution of sICAM-1, sCD44std and sE-cadherin cyst fluid concentrations in cystic lesions of the ovary

Important overlaps were observed in the distribution of the individual cyst fluid concentrations for sICAM-1 and sCD44std. However, the number of cases presenting with cyst fluid concentrations of sICAM-1 or sCD44std >1000 ng/ml, corresponding to the median value ± 2SD found in cystadenomas and luteal cysts, was significantly higher in patients with malignant tumours than in those with benign cystic lesions, including luteal cysts, cystadenomas and dermoid tumours (Fisher’s exact test, P < 0.001 and P = 0.01, respectively).

The most significant differences in the distribution of individual values were observed for sE-cadherin (Figure 1). Overlap between the groups of benign cystic lesions and that of borderline and malignant tumours was limited. Cyst fluid sE-cadherin concentrations were >10 000 ng/ml in 11 of 16 cases of borderline and malignant tumours, but in none of 52 patients with benign cystadenomas or luteal cysts (Table III). The difference was statistically significant (Fisher’s exact test, P < 0.0001). We therefore tested the diagnostic value of the cyst fluid assay of sE-cadherin for the distinction between malignant and benign cystic lesions of the ovary. With a threshold value of 10 000 ng/ml, corresponding to the median value ± 3 SD found in cystadenomas and luteal cysts, the sensitivity of the assay was 69%, its specificity was 100%, its positive predictive value was 100%, and its negative predictive value was 92%.

A statistically significant correlation was observed between the cyst fluid concentrations of sCD44std and sE-cadherin in benign cystadenomas (Spearman correlation test, P = 0.02) and borderline or malignant tumours (Spearman correlation test, P = 0.003), but not in luteal cysts.

For sICAM-1, sCD44std and E-cadherin, there was no statistically significant correlation between concentrations in...
serum and cyst fluid samples obtained in the same patient, irrespective of the type of cystic lesion.

**Discussion**

In our experience, serum concentrations of sICAM-1, sCD44std and sE-cadherin in the serum are of little value for the diagnosis of cystic ovarian tumours. Circulating concentrations of sICAM-1 are higher in patients with ovarian tumours than in healthy subjects, as previously reported in ovarian carcinoma (Giavazzi et al., 1994) and in various other examples of epithelial tumours (Banks et al., 1993; Hyodo et al., 1993). However, our study identifies the fact that serum sICAM-1 concentrations are not significantly different between patients with benign or malignant cystic lesions. It is therefore likely that the increased sICAM-1 concentrations detected in patients with ovarian disease correspond to a non-specific inflammatory reaction.

In contrast to sICAM-1, serum concentrations of sCD44std were comparable in our patients and in healthy subjects. Our experience is in keeping with previous data, showing no increase in the serum concentrations of CD44std and of the CD44v5 and v6 isoforms in patients with ovarian carcinoma (Sliužytė et al., 1995). As for sCD44std, no elevation in serum concentrations of sE-cadherin was noted according to histological type. The absence of any increase in serum concentrations of sCD44std and sE-cadherin in patients with ovarian cancer included in our study contrasts with the usual increase in serum concentrations of these adhesion molecules observed in various other types of malignancies, including cervical, gastric, colon and liver cancer and lymphoma (Banks et al., 1993; Ristamäki et al., 1994; Kainz et al., 1995). The reason for this apparent discrepancy is not clear. A possible cause may be the cystic nature of the tumours included in our study. It has been postulated that the cyst wall acts as a barrier preventing the diffusion of products released by ovarian epithelial cells into the blood (Fleuren et al., 1987; Menczer et al., 1993). This may be interesting to further compare serum concentrations of soluble adhesion molecules in solid and cystic ovarian carcinoma in order to substantiate this hypothesis.

Detectable concentrations of sICAM-1, sCD44std and sE-cadherin were present in all samples of cyst fluids tested in our study. This is in line with previous studies showing the presence of soluble adhesion molecules in the biological fluids located at the contact of tumour cells. Ovarian tumour cells are likely to constitute the main source of the soluble adhesion molecules released into the cyst fluid. This is the case for many membrane glycoproteins used as tumour markers, such as CA125, CA19.9, CA195 and CEA (Montag, 1990; Menczer et al., 1993). However, it cannot be excluded that inflammatory cells, such as lymphocytes and monocyte-macrophages which are present in the cyst wall and in the cyst fluid (Geier and Strecourt, 1981; Trope, 1981), may contribute to the release of sICAM-1 and sCD44std in the cyst fluid.

In our study, we observed no correlation between the serum and cyst fluid concentrations measured in the same patient. This recalls the findings previously reported for other ovarian tumour markers, such as CA125. The absence of correlation observed between serum and cyst fluid concentrations of tumour markers has been interpreted by some authors as a further argument supporting the existence of a structural or functional barrier between intra-cystic epithelial ovarian cells and the blood (Fleuren et al., 1987; Menczer et al., 1993).

In contrast to serum concentrations, cyst fluid concentrations of sICAM-1, sCD44std and sE-cadherin were significantly different in the various types of cystic ovarian lesions included in our study group (P < 0.03). In particular, the mean cyst fluid values of sICAM-1, sCD44std and sE-cadherin were significantly higher in patients with borderline and malignant lesions than in patients with cystadenomas. The increased concentrations of cyst fluid concentrations for CD44std and ICAM-1 observed in ovarian borderline and malignant tumours were concurrent with the increased cellular expression of the corresponding membrane proteins usually observed in these tumours (Cannistra et al., 1995; Uhl-Steidl et al., 1995). In contrast, the highest cyst fluid concentrations of sE-cadherin are observed in cases of borderline and malignant tumours, whereas the lowest are present in benign lesions. This contrasts with previous immunohistochemical results, showing that the apparent expression of E-cadherin is usually lower in cells of borderline and malignant tumours than in cells from cystadenomas (Darai et al., 1997). This may indicate that one of the mechanisms responsible for the apparent decrease in E-cadherin expression at the surface of neoplastic cells could be an increased shedding from the plasma membrane.

The possible clinical relevance of cyst fluid concentrations of soluble adhesion molecules to distinguish between benign and malignant tumours of the ovary was hampered by the large overlaps seen between the ranges measured in each group. This was particularly marked for sICAM-1 and sCD44std, which presented largely overlapping values in the different groups of lesions investigated. However, for sE-cadherin, it is possible to achieve a good distinction between benign and malignant lesions by using a threshold value at 10 000 ng/ml corresponding to the median value in benign cysts ± 3 SD. By using this threshold value, we obtained a sensitivity of 69% with a positive predictive value of 100% and a negative predictive value of 92%. However, these encouraging results must be interpreted with caution due to the limited number of patients included in the present study.

The potential clinical relevance of measuring cyst fluid concentrations of soluble adhesion molecules is enhanced by the popularity of diagnostic procedures based on coelioscopic or laparoscopic examination and including fine-needle aspiration of ovarian cysts (Parker et al., 1992, 1994; Naiman, 1995). Cyst fluid obtained at aspiration may be used to determine...
various markers, including oestradiol, progesterone and CA125, and for cytological examination (Geier and Streeker, 1981; Mencer et al., 1993; Andersen et al., 1995). Several studies have underlined the lack of sensitivity of all these tests for the distinction between benign and malignant lesions (Geier and Streeker, 1981; Mencer et al., 1993; Andersen et al., 1995). Therefore, the determination of cyst fluid concentrations of adhesion molecules, and particularly sE-cadherin, may be considered as an additional tool in the diagnostic management of ovarian cystic masses.

In conclusion, the results of our study suggest that serum concentrations of sICAM-1, sCD44std and sE-cadherin have no diagnostic relevance in the management of cystic epithelial tumours of the ovary. In contrast, the measurement of sE-cadherin concentrations in cystic fluid may be useful in discriminating between benign and malignant cystic tumours.

References

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Adhesion molecules in ovarian tumours