Serum sICAM-1 (soluble intercellular adhesion molecule-1) and M-CSF (macrophage colony-stimulating growth factor) throughout monitoring of 34 non-serous ovarian cancers

N. Callet[1], *M.D.*; **J. Delaunay**[2], *BSc.*; **M. F. Pichon**[2], *D.Pharm, D.Sci*

[1]Service de Gynécologie; [2]Laboratoire d'Oncobiologie, Service de Médecine Nucléaire, Centre René Huguenin de Lutte Contre Le Cancer, Saint-Cloud (France)

Summary

Purpose: Evaluation of serum ICAM-1 (soluble intercellular adhesion molecule-1) and M-CSF (macrophage colony-stimulating growth factor) determinations for monitoring patients with non-serous ovarian cancers.

Methods: ELISA assay of sICAM-1 (cut-off 235 ng/ml) and M-CSF (cut-off 450 pg/ml) in 190 blood samples from 34 patients.

Results: In pre-treatment sera (n=17), sICAM-1 was over the cut-off in 12/17 (70.6%), M-CSF in 14/17 (82.4%) and CA 125 in 12/16 (75%). sICAM-1 was related only to age at diagnosis (p=0.0008). M-CSF was positively correlated to FIGO stage (p=0.04) CA 125 was elevated in 90.9% of adenocarcinomas (p=0.033 vs other). None of the 3 biological markers were related to other clinico-pathological criteria.

Among disease-free patients, higher median concentrations of sICAM-1 and M-CSF were recorded under adjuvant treatment than without (p=0.014, and p=0.08, respectively). After relapse, the highest levels of sICAM-1, M-CSF and CA 125 were observed in progressive disease (46/53, 86.8% (p=0.014), 51/53 96.2% (p=0.008) and 46/52 88.5% (p>0.05), respectively).

Conclusion: In non-serous ovarian cancers, sICAM-1 although elevated in most cases, is not useful for monitoring. Serum M-CSF is a valuable marker when ovarian tumours do not express CA 125.

Key words: ICAM-1; M-CSF (CSF-1); Ovarian cancer.

Introduction

The majority of ovarian carcinomas are serous adenocarcinomas, and for this histological type CA 125 is a well established circulating tumour marker.

Only a part of the other histological types of ovarian carcinomas express CA 125, and there is a need for a marker to monitor such tumours.

The aim of the present work was to study the circulating forms of two biological parameters, known to be expressed in various cancers: sICAM-1 (soluble intercellular adhesion molecule-1) and M-CSF (macrophage colony stimulating factor), which may be of value for this purpose.

ICAM-1 is an adhesion molecule of the immunoglobulin super-family, whose expression is regulated by cytokines (tumour necrosis factor α, interferon γ), and is also up-regulated by high dose γ rays irradiation or by 5-fluorouracil and platinum-based chemotherapies [1, 2]. ICAM-1 is the ligand of lymphocyte function associated antigen-1 (LFA-1, $\beta 2$ integrin) and participates in cell-cell interactions in inflammatory and immune responses. Its expression is necessary for T-cell activation and anti-tumour immune responses in vivo. Few clinical studies on sICAM-1 in human cancer have been published up to now [3-6]. They show with one exception [5] that sICAM-1 is found in higher concentrations in blood, ascites and cyst fluid of cancer patients than in normal subjects or patients with ovarian benign conditions [3-6]. The clinical correlates of sICAM-1 with ovarian cancer at diagnosis or throughout monitoring have not been fully studied. Ferdeghini *et al.* showed that sICAM-1 was unrelated to FIGO stage and histological type of ovarian tumours [5].

Macrophage colony-stimulating factor-1 (M-CSF or CSF-1) was originally defined as a cytokine activating proliferation and differentiation of macrophages from bone marrow. Current data show broader biological activities for M-CSF since this growth factor is involved in infectious, inflammatory and neoplastic diseases [7]. M-CSF acts through a high affinity tyrosine kinase membrane receptor, product of the c-fms oncogene. Three isoforms of M-CSF have been isolated, one associated to the cell membrane and one to the extranuclear matrix, active locally, and another soluble glycoprotein form, active by humoral route. A wide range of non-haematologic cells is known to synthesise M-CSF and its receptor, particularly uterine epithelial cells and cells from normal trophoblasts, both under oestradiol and progesterone control, and also ovarian cancer cells (8-10). The presence of measurable amounts of M-CSF in sera from ovarian cancer patients was originally described by Kacinski [11], and elevated levels of M-CSF in sera or ascites of epithelial ovarian cancer patients were found to be associated with a bad prognosis [12, 14, 15] or recurrences [11, 14, 16-20]. The multiple cellular origins of M-CSF raise the possibility that the concentrations measured in sera of ovarian cancer patients might incorrectly

Revised manuscript accepted for publication October 27, 1999

reflect its synthesis by the tumour, but a recent work by Chambers showed that circulating M-CSF mainly reflects tumour production [12]. In the present work we present the comparative results of sICAM-1, M-CSF and CA 125 assays in pretreatment sera and throughout monitoring of 34 ovarian cancer patients, selected for their less frequent and particular histological type.

Patients and Methods

Patients

We retrospectively measured sICAM-1 and M-CSF in 190 sera kept deep frozen in our blood bank and drawn from 34 patients followed-up and treated at the Centre Rene Huguenin between June 1986 and September 1997. Their mean age at diagnosis was 56.7±10.7 years. CA 125 was measured in 173 blood samples. Pretreatment sera were available for 17 patients, and their median follow-up time was 4.3 years (interquartile range (IQR) 3.9 years).

All patients had a non-serous ovarian carcinoma: 3 clear cell carcinomas, 6 endometrioid carcinomas, 6 mucinous carcinomas, 10 non-serous non-mucinous adenocarcinomas with different degrees of differentiation, 4 papillary carcinomas, 1 mixed mesodermic and mullerian tumour, 3 granulosa cell tumours. Ten patients in this group never raised their serum CA 125 above cut-off.

Patients were clinically staged according to FIGO (International Federation of Gynecology and Obstetrics) classification. Ten (29.4%) patients were stage I, 2 (5.9%) stage II, 18 (52.9%) stage III, four (11.8%) stage IV. Initial symptoms were ascites in 14 cases (41.2%), abdominal or pelvic pain in 23 cases (67.6%), digestive problems in six cases (17.6%), perception of a tumour in 24 cases (70.6%), bleeding in three cases (8.8%).

The initial evaluation included a full clinical examination, abdominal and pelvic ultrasonography, abdominal and pelvic CT scan, chest X-rays, routine blood tests and serum CA125 assays when applicable. All the patients studied were without impaired renal function. Patients bearing other cancers, autoimmune disease, and chronic inflammatory diseases were also excluded.

All patients had a first laparotomy, 27 of them had completed first line chemotherapy, eight patients also had adjuvant radiotherapy and two patients hormone therapy by progestogens. Only five patients had a second-look after first line chemotherapy.

At the endpoint of the study, 13 patients (38.2%) died from ovarian cancer.

Methods

All assays were performed in duplicate on serum samples.

1. CA 125 assays were performed by an IRMA (Elsa CA 125 II, Cis Bio International, Gif sur Yvette, France). Quality control was ensured by using two levels of control sera in each assay, and by participation to the Oncocheck™ European program.

2. sICAM-1 assays were performed by an ELISA (Parameter, R & D Systems, Abingdon, UK). Quality control was monitored by using the kit's control serum. This technique has a sensitivity of < 0.35 ng/ml. Our intra-assay precision was 4.63% and inter-assay precision 4.0%.

3. M-CSF assays were perfomed by using the Quantikine ELISA technique (R & D Systems). As no control serum was provided in the package, human serum from a normal subject was used for quality control. This technique has a sensitivity of < 40 pg/ml, with an intra-assay precision of 9.5% and an inter-assay precision of 11.2%.

4. Statistical calculations were made by using Statview v. 5.0 (Cary, NC, USA) and ROC (receiver operating characteristic) curve analysis was done by using MedCalc v. 4.3 (Mariakerke, Belgium). Since the groups compared were generally small, we only used non-parametric statistics, with a level of significance of 0.05 (2-tail).

Results

1. Cut-off values for sICAM-1 and M-CSF.

To delineate dichotomic groups for statistical analyses, we calculated their respective cut-off by ROC curve analysis. Forty-five sera from normal female subjects were assayed for sICAM-1 and M-CSF. The ROC curve analysis was performed with 34 corresponding results from sera taken at random in our ovarian cancer group. Figures 1 and 2 show the ROC curves for sICAM-1 and M-CSF, respectively. For sICAM-1 the best fit was 235 ng/ml, corresponding to 64.7% sensitivity, 80.0% specificity (area under ROC curve 0.758). The normal values from 131 normal male and female subjects given in the package insert are 210.6 ng/ml (mean), 114.7-306.4 ng/ml (range±2SD). For M-CSF, the cut-off obtained was 450 pg/ml, with a sensitivity of 97.1% and a specificity of 73.3% (area under ROC curve 0.927). The normal values given in the package insert (n=40 healthy individuals) are 670 pg/ml (mean) 253-1,715 pg/ml (range).

2. ICAM-1 and M-CSF in pretreatment sera (n=17).

Results above cut-off were found in 12/17 (70.6%), 14/17 (82.4%), and 12/16 (75.0%) of sICAM-1, M-CSF and CA 125, respectively. Median and interquartile ranges (IQR) were 274.0 ng/ml (72.5) for sICAM-1, 638.0 pg/ml (321.0) for M-CSF, and 277.0 U/ml (1,639.0) for CA 125. The median concentrations of sICAM-1 and M-CSF in the 17 pretreatment sera differed significantly from those of 45 normal subjects ($p=0.0006$ and $p<0.0001$, respectively).

Circulating levels of CA 125, sICAM-1 and M-CSF were compared together, and with the clinical and histological criteria of ovarian cancer (age, menopausal status, type of clinical symptoms at diagnosis, associated pathologies, existence of previous cancers, histological classification, FIGO staging). No correlations were found between serum concentrations of sICAM-1 or M-CSF with CA 125 concentration in pretreatment sera. sICAM-1 was found to be positively correlated with the age of the patients ($p=0.008$). None of the biological markers were correlated with clinical symptoms at diagnosis: presence of ascites, pain, or digestive problems, presence of a tumour mass, bleeding. Neither was there any correlation with a history of benign thyroid pathologies (n=9) or thyroid papillary carcinoma (n=1), nor with other previous cancers (n=8). Serum M-CSF was found to be positively correlated with FIGO stage ($p=0.04$). The concen-

tration of sICAM-1 or M-CSF was not significantly associated with the histological type of the tumours, even when comparing adenocarcinomas versus other histological types. However, a higher frequency of elevated CA 125 (90.9%) was found in patients bearing adenocarcinomas versus other histological types (p=0.033, χ^2 tests).

Kaplan-Meier univariate survival curves showed non-significant differences in overall survival between positive and negative sICAM-1, M-CSF, or CA 125 groups.

3. Comparison of circulating sICAM-1, M-CSF and CA 125 according to post surgical residues (n=19).

The 3 biological markers were measured before and 39±28 days after surgery for 19 patients, 9 of them devoid of macroscopical and microscopical tumour residue, 10 with histologically proven residue. Median concentrations of sICAM-1, M-CSF and CA 125 were lower in sera from patients without residual disease than in the opposite group (Table 1). Differences in median concentrations were significant for sICAM-1 (p=0.016), and CA 125 (p=0.018).

4. Distribution of sICAM-1 and M-CSF levels according to clinical states during ovarian cancer monitoring (n=189).

The clinical states encountered during monitoring of our series of ovarian cancers were classified as follows: pretreatment, follow-up with or without adjuvant treatment, treated recurrences subdivided according to responses: regression, stable disease, progression. Each of the 190 blood samples drawn during monitoring was quoted according to the above categories. The distribution according to the different clinical categories is summarised in table 2. All the patients of this series had at least one serum M-CSF level above cut-off throughout monitoring.

When considering overall results, the concentrations of serum sICAM-1 and M-CSF were found to be correlated with each other (n=190, p<0.0001). sICAM-1 and M-CSF were also correlated with CA 125 (n=174, p<0.001 for both). Median concentration of the three parameters differed significantly according to the six different clinical states (p<0.0001) (Fig. 3). Serum changes before and after initial treatment were also studied. Significant differences were found only for CA 125 (p=0.0002), its level falling under normal range after removal of the tumour.

When comparing pretreatment, disease-free during follow-up and recurrences as single groups, significant differences remained (p=0.0003 for sICAM-1, 0.015 for M-CSF, and p<0.0001 for CA 125). Patients with progressive disease had elevated median concentrations of all parameters. When analysing the median concentrations observed within the three categories of treatment responses, no significant differences were found for sICAM-1 distributions (p=0.078), but median concentrations of M-CSF or CA 125 differed significantly (p<0.0001 for both), with higher concentrations in sera from patients with progressive disease.

Comparison of serum concentrations of the three parameters during follow-up without relapse led to significant differences for sICAM-1 and M-CSF between patients with and without adjuvant treatment (p=0.014 and p=0.008, respectively). In the same groups non-significant differences were obtained for CA 125.

Analysis of patient medical records during monitoring showed that serum M-CSF follows generally the pattern of CA 125 when the latter is expressed. However, differences between the profiles can be observed in some instances. Discordant results of serum M-CSF (elevation in 26/28 cases) with reference to CA 125 or to clinical states were analysed in 13 patients with more than four serial serum marker determinations (total of comparisons: 114). Fourteen out of 28 discordances (50%) corresponded to samples drawn during chemotherapy. Such an increase of M-CSF was reported by Kimura during chemotherapy of acute leukemias [13]. We also recorded 5/28 (17.9%) transient elevations of M-CSF during infectious complications. One case was explained by the appearance of a breast tumour, and seven cases remained unexplained through medical records. Figure 4 shows an example of serial sample assays throughout follow-up of a mucinous ovarian cancer.

Table 1. — *Circulating levels of sICAM-1, M-CSF and CA 125 according to postsurgical tumour residues.*

	sICAM-1 ng/ml Median (IQR)*	M-CSF pg/ml Median (IQR)	CA 125 U/ml Median (IQR)
No residue	211.0 (47.7)	510.0 (329.0)	41.0 (85.0)
Proven residue	258.5 (81.0)	829.0 (463.0)	234.0 (1,686.0)
Significance (Mann-Whitney test)	p = 0.016	p = 0.063	p = 0.018

* IQR = interquartile range

Table 2. — *Distribution of sICAM-1, M-CSF and CA 125 according to clinical states throughout monitoring of 34 non-serous ovarian cancers.*

	sICAM-1 ng/ml Median (IQR)* % > 235 ng/ml	M-CSF pg/ml Median (IQR) % > 450 pg/ml	CA 125 U/ml Median (IQR) % > 35 U/ml
Pretreatment (n=17)	242.0 (78.5)	585.0 (350.5)	250.0 (1,518.2)
	13/23 (56.5%)	17/23 (73.9%)	12/17 (70.6%)
Disease-free			
without treatment (n=58)	229.5 (78.0)	500.0 (340.0)	14.0 (39.2)
	28/58 (48.3%)	37/58 (63.8%)	16/55 (29.1%)
with adjuvant treatment (n=15)	297.0 (108.7)	723.0 (411.0)	51.0 (81.2)
	11/15 (73.3%)	14/15 (93.3%)	8/13 (61.5%)
Recurrences			
stable disease (n=20)	261.0 (88.0)	712.0 (241.0)	18.0 (24.5)
	13/20 (65.0%)	17/20 (85.0%)	5/16 (31.2%)
regression (n=21)	277.0 (110.0)	479.0 (212.0)	47.0 (55.0)
	18/21 (85.7%)	13/21 (61.9%)	11/21 (52.4%)
progression (n=53)	289.0 (100.0)	849.0 (579.0)	209.5 (975.5)
	46/53 (86.8%)	51/53 (96.2%)	46/52 (88.5%)

* IQR = interquartile range

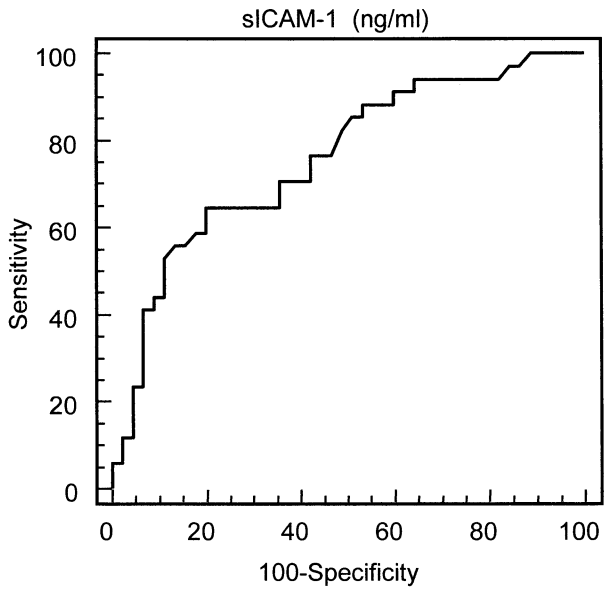

Figure 1. — ROC (receiver operating characteristic) curve analysis of serum ICAM-1. Cut-off 235 ng/ml.

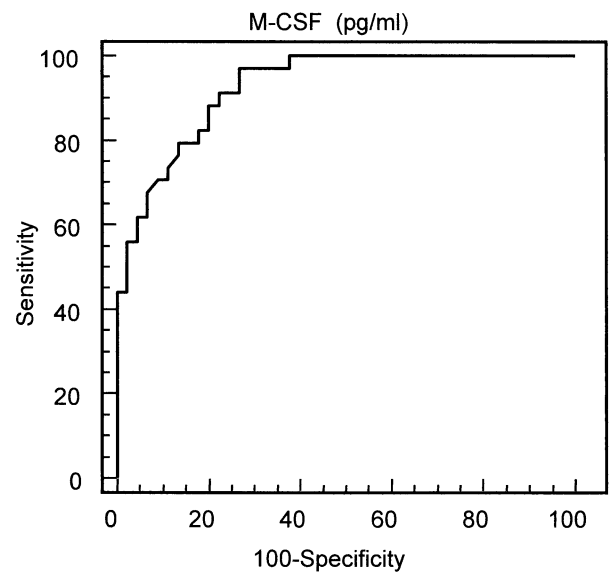

Figure 2. — ROC curve analysis for serum M-CSF. Cut-off 450 pg/ml.

Discussion

Pretreatment CA 125 was elevated in 10/11 (90.9%) of the non-serous adenocarcinomas of our series and the serum level varied according to tumour recurrences and responses to treatment as classically described.

The single significant relationship found for sICAM-1 in pretreatment sera was with the age of the patients at diagnosis. The increase of circulating sICAM-1 with age was reported by Rodhe *et al.* in a group of men studied for cardiovascular risk factors [23]. During monitoring of our group of patients, circulating concentrations of ICAM-1 were found to be quite constantly over cut-off,

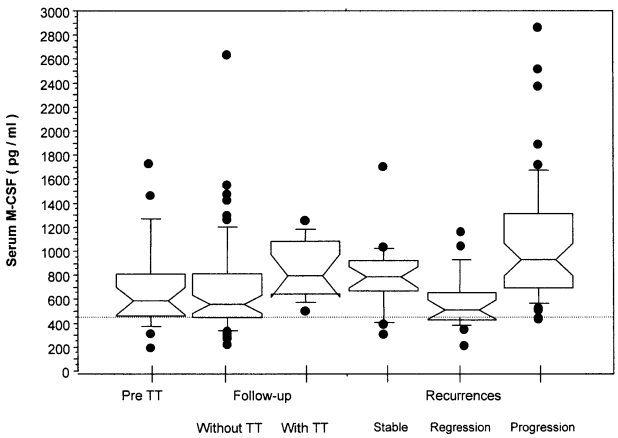

Figure 3. — Serum M-CSF distribution according to clinical states (n=190). TT=treatment.

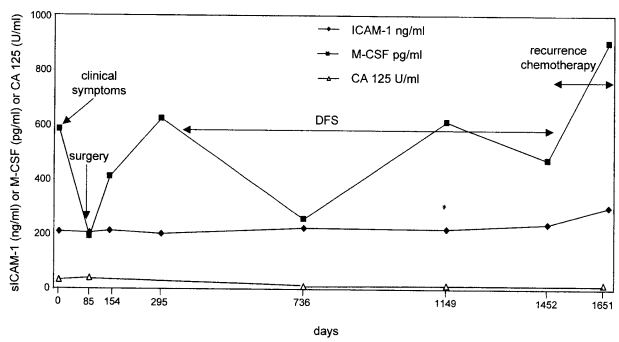

Figure 4. — Example of serial assays of ICAM-1 M-CSF and CA 125 throughout follow-up of an ovarian mucinous adenocarcinoma. DFS = disease-free survival.

but never very elevated. Levels were under cut-off only in sera from disease-free patients without adjuvant treatment, or without residual disease after surgery. Frequently elevated sICAM-1 in ovarian cancer has already been reported by several authors as a result of inflammatory or immune mechanisms [3, 4, 6]. However, the work by Giavazzi *et al.* [4] suggests that sICAM-1 can also be released from the tumour itself. We sought to confirm this hypothesis by searching for a postoperative decrease of sICAM, but did not find significant differences in median concentrations before and after surgery, possibly because of the small size of our series. Like Ferdeghini *et al.* we found no relathionship between sICAM-1 and histological types nor with FIGO stages [5]. Overall, it appears that circulating levels of this biological marker provide little information regarding the evolution of non-serous ovarian cancers.

The variations of serum M-CSF recorded in our series are in accordance with previous studies showing that this growth factor was elevated in ovarian cancers, and followed changes in disease status [11, 13, 16-20]. We found serum M-CSF to be constantly elevated, from the outset of the disease (73.9% over cut-off) and throughout

its evolution (from 61.9 to 96.2% according to disease states), with a maximum proportion above cut-off during progressive disease. We also found a positive relationship of serum M-CSF with tumour load, as expressed by FIGO stages.

In accordance with the results of Gadducci *et al.* [20] we found the expression of M-CSF to be independent of histological tumour types of ovarian cancers. Noteworthy, serum M-CSF was elevated in the ten tumours which did not express CA 125. In a recent work, Keshava and coauthors pointed out an overexpression of CSF-1 (M-CSF) by cultured normal granulosa cells leading to cell proliferation and tumorigenesis [9]. In vivo, such a trend was previously reported by Woolas with 3/5 positive sera from patients with stage I granulosa cell tumors [21]. Similarly, we recorded 3/3 relapse-free patients with granulosa cell tumors with an elevated pretreatment concentration of M-CSF. During follow-up two out of the three showed serum M-CSF below normal range and the remaining was close to cut-off (481 pg/ml) 2.5 years after surgery.

Three groups reported a prognostic value for M-CSF [12, 14, 15]. Due to the small size of our group made up of infrequent tumour types, we could not prove prognostic values for the three biological parameters.

Overall, the monitoring of tumours which do not express CA 125 by serial M-CSF measurement is feasible, provided that eventual likely "non-specific" causes of elevation such as infectious disease are identified. Another drawback is the differing pattern from CA 125 during chemotherapy courses, due to the complexity of the origins of serum M-CSF, released by tumour and bone marrow cells lysis, and up-regulated before haematological recovery [13].

Conclusion

To conclude, although mostly above-normal range during the evolution of ovarian cancer, the level of expression of sICAM-1 shows a narrow range of variations between different clinical situations, and consequently is hardly effective in ovarian cancer monitoring. On the contrary, assays of circulating M-CSF are a valuable tool for ovarian cancer monitoring, but only when they do not express CA 125.

Acknowledgements

This work was supported by a grant from the Ligue Nationale Contre Le Cancer, Comité des Hauts de Seine. The authors thank Ms B. Bicknell for her help in checking the manuscript.

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Address reprint requests to:
MARIE-FRANCE PICHON, M.D.
Centre René Huguenin
35, rue Dailly
92 210 Saint-Cloud (France)