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Serum adhesion molecules and interleukin-2 receptor as markers of tumour load and prognosis in advanced cutaneous melanoma

M.-S. Vuoristo[a,*], S. Laine[b], H. Huhtala[c], L.-M. Parvinen[d], M. Hahka-Kemppinen[e], M. Korpela[f], E. Kumpulainen[g], P. Kellokumpu-Lehtinen[a]

[a]*Department of Oncology, University of Tampere and Tampere University Hospital, POB 2000, Fin-33521 Tampere, Finland*
[b]*Department of Clinical Microbiology, Tampere University Hospital, Tampere, Finland*
[c]*School of Public Health, University of Tampere, Tampere, Finland*
[d]*Department of Oncology, Turku University Hospital, Finland*
[e]*Department of Oncology, Helsinki University Central Hospital, Finland*
[f]*Department of Oncology, Oulu University Hospital, Finland*
[g]*Department of Oncology, Kuopio University Hospital, Finland*

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Abstract

Cell adhesion molecules are cell surface glycoproteins that may act as mediators in the metastatic process. Soluble interleukin-2 receptor (sIL-2R) is an immunological marker that may also serve as an indicator of tumour progression. Normal and neoplastic cells are capable of releasing these molecules into circulation. We evaluated the association between pretreatment serum levels of soluble intercellular adhesion molecule 1 (sICAM-1), vascular cell adhesion molecule 1 (sVCAM-1) and sIL-2R and metastases and survival in 50 patients with advanced melanoma. The patients with liver and/or bone metastases had significantly higher sICAM-1 levels than those with soft tissue and/or lung involvement ($P=0.002$). In addition, there was a trend towards higher sIL-2R levels in patients with more metastatic sites ($P=0.07$). In univariate analysis, the number of metastatic sites ($P=0.0001$, odds ratio (OR) 3.0, 95% confidence interval (CI): 1.7–5.3), the metastatic site ($P=0.01$, OR 2.3, 95% CI: 1.2–4.4) and the levels of sICAM-1 ($P=0.011$, OR 2.5, 95% CI: 1.2–5.0), sVCAM-1 ($P=0.036$, OR 2.1, 95% CI: 1.0–4.3) and sIL-2R ($P=0.0016$, OR 3.0, 95% CI: 1.5–6.0) were found to be statistically significant prognostic factors for survival. In multivariate analysis, the number of metastatic sites was the dominant prognostic indicator. After it was excluded from the analysis, the sIL-2R level and the metastatic site were found to be significant. It can be concluded, that high sICAM-1 levels suggest liver metastases and sIL-2R seems to serve as a marker of tumour load in metastatic melanoma. Furthermore, the sIL-2R level appears to add to clinical data predicting the patient's outcome.

Keywords: Metastatic melanoma; Prognosis; sICAM-1; sIL-2R; sVCAM-1; Tumour load; Tumour marker

1. Introduction

The clinical course of metastatic melanoma varies considerably with survival ranging from a few months to several years. Although the extent and the location of metastases are the crucial prognostic determinators, serum biomarker measurements could aid in assessing the prognosis of an individual patient more accurately. Cell adhesion molecules are cell membrane glycoproteins that allow cells to adhere to each other and to the extracellular matrix [1]. In cancer patients, the process of successful metastasis is complex and requires tumour cells to possess decreased adhesive interactions with the surrounding cells and extracellular matrix at some points in the metastatic cascade and increased adhesive interactions at other times [2].

Intercellular adhesion molecule 1 (ICAM-1) functions in T-cell mediated host defence mechanisms and in inflammatory processes. ICAM-1 binds to the ligands leucocyte function associated antigen 1 (LFA-1) and Mac-1 that are both expressed on leucocytes [1]. ICAM-1 is expressed by several cell types including leucocytes and endothelial cells. Within the immune system, ICAM-1 is expressed on cells of the monocyte-macrophage lineage, on B-lymphocytes, on plasma cells and

* Corresponding author. Fax: +358-3-247-3009.
E-mail address: meri-sisko.vuoristo@tays.fi (M.-S. Vuoristo).

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on both activated and memory T-cells [1]. Interestingly, melanoma cells may also express ICAM-1. A positive correlation has been observed between ICAM-1 expression on the primary tumour and the risk of metastatic disease, and metastatic lesions have been shown to have a higher expression than primary tumours [3,4]. Cells are capable of secreting soluble ICAM-1 (sICAM-1) into circulation. The regulation and significance of sICAM-1 have not yet been fully elucidated, but sICAM-1 released from melanoma cells may protect them from T-cell mediated lysis [1]. Elevated levels of sICAM-1 have been reported in melanoma patients, in advanced stage patients in particular [5–11].

Compared with the wide expression of ICAM-1, the expression of vascular cell adhesion molecule 1 (VCAM-1, previously referred to as INCAM-110) is more restricted [12–13]. VCAM-1 is expressed by endothelial cells, dendritic cells, some tissue macrophages, renal parietal epithelial cells, synovial lining cells and reactive mesothelial cells [13]. In addition, benign nevi and melanoma cells have been reported to express VCAM-1 [14]. Local inflammation leads to increased expression of VCAM-1 on vascular endothelium which contributes to the encapture of leucocytes from the circulation through the leucocyte ligand $\alpha 4\beta 1$ integrin [15]. Melanoma cells have been shown to adhere to the endothelium via VCAM-1, which may facilitate haematogeneous metastasis [16]. Elevated levels of VCAM-1 in serum (sVCAM-1) have been observed in some [17], but not all of the previous reports on melanoma [6,8].

Interleukin-2 (IL-2) plays a pivotal role in regulating immunological functions. Activated T cells secrete a truncated form of IL-2R into the circulation as a soluble molecule (sIL-2R) [18]. Over time, accumulating evidence has shown that the biological effects of IL-2R are more complex than simply mediating T-cell growth. Depending on conditions, IL-2R signals may also promote cell survival, effector function and apoptosis [19]. IL-2R is also expressed on non-lymphoid cells, such as oligodendrocyte progenitors, keratinocytes, fibroblasts, endothelial cells and some neoplastic cells [20]. Melanoma cell lines have been shown to express IL-2R and produce functional IL-2 which may play a role in the autocrine stimulation of melanoma cells [20].

sIL-2R may be immunosuppressive by acting as an antagonist of IL-2-mediated cell responses [18]. It has been shown that the addition of natural or synthetic sIL-2R into *in vitro* IL-2 functional assays inhibits the stimulatory effects of exogenously added IL-2 [18]. sIL-2R has been reported to be associated with disease progression and prognosis in haematological malignancies, while in solid cancers its clinical significance is less clear [18,21]. Elevated levels of sIL-2R have been reported in melanoma, even in early stage disease [22–25]. Some investigators have reported a correlation between the sIL-2R level and melanoma progression [22,24].

The purpose of the present study was to investigate the association between the pre-treatment levels of sICAM-1, sVCAM-1 and sIL-2R and metastatic site and tumour load and whether the marker levels could aid in distinguishing different prognostic groups in advanced melanoma.

2. Patients and methods

2.1. Patients and treatment

We included in the study 50 patients with advanced (American Joint Committee on Cancer (AJCC) stage IV) cutaneous melanoma who were to be treated with chemotherapy and α-interferon (IFN) during 1996–1999 in a Finnish randomised multicentre study which is still open for accrual. The male/female ratio was 31/19 and the median age 62 years (range 25–75 years). 26 patients (52%) had cutaneous, subcutaneous, nodal and/or lung metastases exclusively, while 24 (48%) patients had liver and/or bone involvement with or without other metastases. Of these, 20 patients had liver metastases. None of the patients had brain metastases. 9 (18%) patients had one metastatic site, 28 (56%) had two sites, and 13 (26%) had three or more sites.

The treatment protocol had four arms which all contained chemotherapy and αIFN. The arms were either dacarbazine (250 mg/m^2 intravenous (i.v.) days 1–5) or DOBC (BOLD) regimen (dacarbazine 200 mg/m^2 i.v. days 1 to 5, vincristine 1 mg/m^2 i.v. days 1 and 4, bleomycin 15 mg i.v. days 2 and 5 and CCNU 80 mg orally day 1) plus either recombinant or natural αIFN subcutaneously (s.c.). The dose of αIFN was 3×10^6 IU daily starting on day 8, for 6 weeks and 6×10^6 IU three times weekly thereafter. The chemotherapy cycle was 4 weeks.

2.2. Analysis of blood samples

Blood samples for analysis of serum concentrations of sIL-2R, sICAM-1 and sVCAM-1 were collected from patients before treatment and stored at −70°C until analysis. sIL-2R was analysed by Quantikine® human IL-2 sRα Immunoassay (R&D Systems, Minneapolis, MN, USA) which has a sensitivity of 6 pg/ml. sICAM-1 was analysed with Parameter® human sICAM-1 (R&D Systems) with a sensitivity of 0.35 ng/ml. sVCAM-1 was measured with Parameter® human sVCAM-1 (R&D Systems) which allows detection of 2 ng/ml.

2.3. Statistical analysis

The Mann–Whitney and the Kruskal–Wallis tests were used to study the differences in marker concentrations between groups. The Chi square test was used to

Table 1
Cox univariate analysis of prognostic factors in advanced melanoma

Variable	Categories compared	Hazard ratio (95% confidence interval)	*P* value
Gender	Male versus female		0.75
Age (years)	<62 versus $\geqslant 62$ years		0.65
Metastatic site	Liver/bone versus other	2.3 (1.2–4.4)	0.01
Number of sites	1 versus 2 versus $\geqslant 3$	3.0 (1.7–5.3)	0.0001
	1 versus 2	3.2 (1.1–9.4)	0.03
	1 versus $\geqslant 3$	9.4 (2.9–31.0)	0.0002
	2 versus $\geqslant 3$	2.7 (1.3–5.6)	0.01
sIL-2R	$<$ versus $\geqslant 1558$ pg/ml	3.0 (1.5–6.0)	0.0016
sICAM-1	$<$ versus $\geqslant 407$ ng/ml	2.5 (1.2–5.0)	0.011
sVCAM-1	$<$ versus $\geqslant 768$ ng/ml	2.1 (1.0–4.3)	0.036

sIL-2R, soluble interleukin-2 receptor; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1.

analyse the association between the metastatic site and the number of metastatic sites. Survival was calculated from the start of the treatment to the date of death or to the last follow-up date. For each marker, we analysed the mean and the quartiles as cut-off points with the Kaplan–Meier method (log-rank test) in order to define the cut-off value that would best discriminate short and long survival. Subsequently, Cox univariate and multivariate regression analyses were performed to study further the impact of variables on survival.

3. Results

The median level of sICAM-1 was 297 ng/ml and the range was 147–1340 ng/ml. For sVCAM-1, it was 626 ng/ml with a range of 265–1979 ng/ml and for sIL-2R, it was 1261 pg/ml with a range of 637–7613 pg/ml. There were no differences in the marker levels according to gender or age (data not shown).

The patients with liver and/or bone metastases had significantly higher concentrations of sICAM-1 than patients with soft tissue and/or lung involvement exclusively (370 ng/ml versus 267 ng/ml, respectively, $P=0.002$). All the patients ($n=7$) with the sICAM-1 level above 500 ng/ml and 85% (11/13) of the patients with the level above 400 ng/ml had liver involvement. The differences in sIL-2R or sVCAM-1 levels were not statistically significant (1418 pg/ml versus 1168 pg/ml, $P=0.12$, and 642 ng/ml versus 626 ng/ml, $P=0.91$, respectively). There was a strong association between the metastatic site and the number of metastatic sites ($P=0.002$). Eight percent of the patients with soft tissue and/or lung metastases and 46% of the patients with liver/bone involvement had three or more involved sites.

There was no statistically significant association between the marker levels and the number of metastatic sites. However, the patients with three or more involved sites had higher concentrations of sIL-2R than the patients with only one involved site (1421 pg/ml versus 1021 pg/ml, $P=0.07$). All the patients ($n=14$) with the sIL-2R level above 1500 pg/ml had two or more involved sites. The levels of sICAM-1 or sVCAM-1 according to the number of metastatic sites were not significantly different ($P=0.29$ and $P=0.75$, respectively).

After a median follow-up of 9 months, 39 (78%) patients had died. The median survival for the entire group was 9 months (range 2 weeks to 42 months). With the current patient accrual, there were no statistically significant differences in survival between the four treatment arms (data not shown). Using univariate analysis, the metastatic site, the number of metastatic sites and all the tested markers predicted survival with statistical significance (Table 1). The patients with one metastatic site survived 18 months, those with two sites, 9 months, and those with three or more sites, 7 months. The median survival of the patients with liver and/or bone metastases was 7 months, while it was 12 months for those with other metastases. The patients with sIL-2R

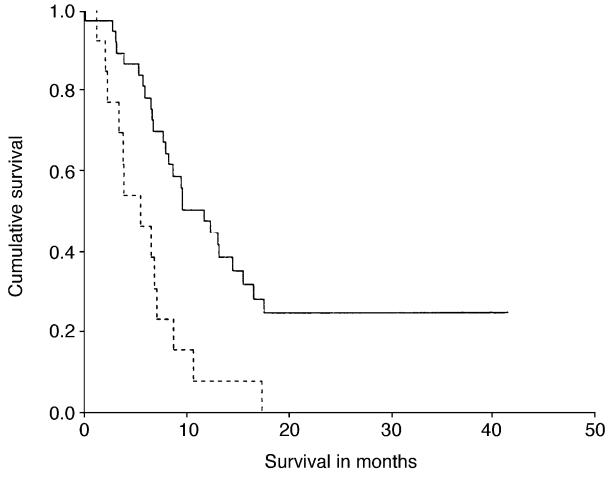

Fig. 1. Survival according to soluble interleukin-2 receptor (sIL-2R) in patients with advanced melanoma. Solid line: sIL-2R <1558 pg/ml; dotted line: sIL-2R $\geqslant 1558$ pg/ml. $P=0.0009$.

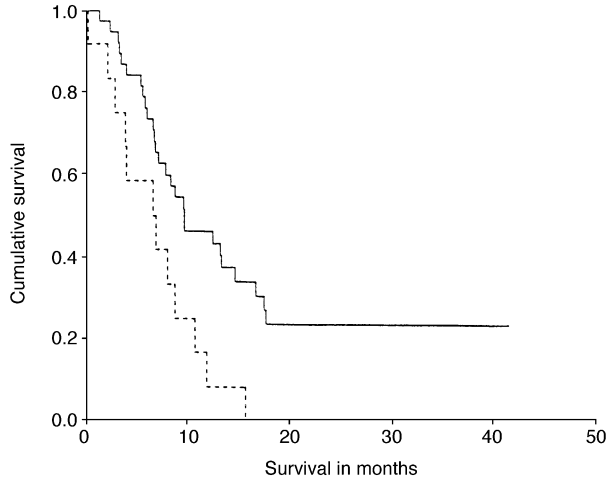

Fig. 2. Survival according to soluble intercellular adhesion molecule 1 (sICAM-1) level in patients with advanced melanoma. Solid line: sICAM-1 <407 ng/ml; dotted line: sICAM-1 $\geqslant 407$ ng/ml. $P=0.009$.

values below 1558 pg/ml (the mean), had a median survival of 12 months compared with only 6 months for the patients with higher levels (Fig. 1). The median survival for the patients with sICAM-1 and sVCAM-1 concentrations below a cut-off point at upper quartile was 10 months, and for those with higher levels, 7 and 4 months, respectively (Figs. 2 and 3). Gender and age were not prognostically significant.

In multivariate analysis, only the number of metastatic sites was a statistically significant prognosticator ($P<0.001$, odds ratio (OR) 3.2, 95% confidence interval (CI): 1.1–9.4 when the patients with one or two sites were compared; OR 9.4, 95% CI 2.9–31.0 when the patients with 1 or 3 or more sites were compared). After excluding the number of metastatic sites, sIL-2R concentration ($P=0.004$, OR 2.8, 95% CI: 1.4–5.6) and metastatic site ($P=0.025$, OR 2.1, 95% CI: 1.1–4.0) reached statistical significance.

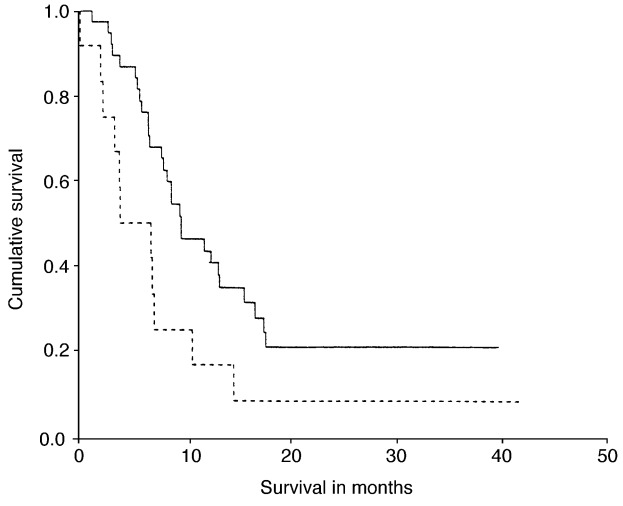

Fig. 3. Survival according to vascular cell adhesion molecule 1 (sVCAM-1) level in patients with advanced melanoma. Solid line: sVCAM-1 <768 ng/ml; dotted line: sVCAM-1 $\geqslant 768$ ng/ml. $P=0.03$.

4. Discussion

In previous studies, serum S-100ß has proved a prognostic tumour marker in melanoma [26–28]. In order to study the clinical value of sICAM-1, sVCAM-1 and sIL-2R as tumour markers in metastatic melanoma, we analysed the association between pretreatment levels of the markers and the location and the extent of metastases and survival.

In the present study, the patients with liver and/or bone metastases had significantly higher sICAM-1 levels than those with soft tissue and/or lung involvement only. The majority (83%) of the former group had liver metastases. The higher sICAM-1 concentration in patients with liver metastases is in accordance with previous studies [4,25]. Harning and colleagues, however, did not find a correlation between sICAM-1 and location or extent of metastases in 18 patients with disseminated melanoma [10]. Mononuclear cells, endothelial cells, keratinocytes, hepatocytes and melanoma cells are capable of secreting sICAM-1 into circulation [1]. In the present study, the malignant cells in the liver are probably the source of sICAM-1, rather than the normal hepatocytes. In hepatocellular carcinoma, it was observed that the expression of ICAM-1 in cancer cells was significantly higher than in normal liver cells [29]. sICAM-1 shed from melanoma cells is able to inhibit non-major histocompatibility complex (MHC)-restricted cytotoxicity mediated by natural killer and lymphokine-activated killer cells as well as MHC-restricted specific T- cell/melanoma interactions [1].

sICAM-1 measurements could be used during follow-up of melanoma patients for screening of liver metastases and for guiding diagnostic imaging. The relatively small study population does not allow us to set up a definite threshold level. It seems, however, that levels above 400 ng/ml suggest the presence of liver metastases. In the present study, only patients with cutaneous metastatic melanoma were included, but sICAM-1 may be practical also in ocular melanoma where the liver is the primary target organ for metastases. Recently, a strong ICAM-1 expression in ocular melanoma cells has been reported [30,31]. The ICAM-1 expression on the primary tumour was increased in larger tumours, but it was not an indicator of concurrent metastases [31]. Other investigators observed that lower rather than increased ICAM-1 expression in the primary tumour implied an increased risk for developing metastases in uveal melanoma [30]. To our knowledge, there are no published data on sICAM-1 concentrations in patients with ocular melanoma.

We observed that the pretreatment level of sICAM-1 was a significant prognostic factor for survival by univariate analysis. Elevated sICAM-1 levels have also been found to correlate with short survival in metastatic melanoma in previous studies [9,10,17], but opposing results have been reported in a recent study [11].

In the present study, we did not observe an association between the concentration of sVCAM-1 and the amount or location of metastases. Despite that, sVCAM-1 was a significant prognostic marker for survival in univariate analysis with higher levels predicting shorter survival. Recently, it was reported that sICAM-1, sVCAM-1 and lactate dehydrogenase (LDH) were significant prognostic factors in metastatic melanoma in univariate analysis, while sVCAM-1 and LDH were also significant in multivariate analysis [17]. Melanoma cells may adhere to the endothelium via VCAM-1 and establish metastases [16]. However, the expression of VCAM-1 has not been correlated with the thickness of primary tumour or shown a differential distribution in primary and metastatic lesions [14]. Furthermore, downregulation of VCAM-1 in endothelial cells in the tumour has been proposed as a potential mechanism of immune escape for melanoma cells [32]. Thus, further studies at the molecular and clinical level are needed to clarify the role of VCAM-1 and sVCAM-1 in melanoma.

In the present study, the patients with elevated sIL-2R had more metastatic sites than those with lower levels. Even if the sample size is rather small, it seems that the levels above 1500 pg/ml imply multiple involved sites in melanoma. As opposed to our results, Ostenstad and colleagues did not find a connection between sIL-2R and tumour burden in 33 patients with metastatic melanoma [23]. The positive association between the sIL-2R level and the number of involved sites found in the present study implies that melanoma cells may largely be the source of sIL-2R. Melanoma cell lines have been shown to express a functional IL-2R and a direct involvement of IL-2 in the stimulation of growth of melanoma cells has been shown [20,33]. This could have implications with regard to IL-2 therapy. Exogenously added IL-2 may disrupt the autocrine loop and interfere with the growth regulation of melanoma [20]. Excessive IL-2 may act as a growth inhibitory factor which could be one reason why high dose i.v. bolus IL-2 therapy appears to be more effective than lower dose regimens, even if the optimal dose and schedule of IL-2 in metastatic melanoma remains a topic of controversy [34]. However, the expression of functional IL-2R in melanoma cells is much less than that observed in activated peripheral blood lymphocytes [20]. In theory, shedding of sIL-2R from melanoma cells could also imply growth restriction of melanoma through the loss of a stimulatory signal. This, however, seems unlikely, since high levels of sIL-2R suggested short survival in the present study. Rather, shedding of sIL-2R from melanoma cells could mean an attempt to block IL-2-mediated T-cell cytotoxicity towards melanoma cells. Activated T-cells could also release sIL-2R as a marker of immune response against melanoma. Strong expression of IL-2R in tumour-infiltrating lymphocytes isolated from patients with various solid tumours, including melanoma, has been reported [35]. The present setting does not allow us to draw definite conclusions on the origin of sIL-2R.

In the present study, the sIL-2R level was a significant prognostic factor for survival in univariate analysis. In multivariate analysis, the tumour load was the dominant prognostic factor followed by sIL-2R and the anatomical site of metastatic involvement. Previously, sIL-2R has been reported to correlate with disease progression in melanoma in several studies [11,22,24], but the data on sIL-2R as a prognostic determinator for survival are sparse. In one study, no association between sIL-2R and survival in metastatic melanoma was observed [23].

It can be concluded that high sICAM-1 levels suggest liver metastases and sIL-2R seems to serve as a marker of tumour load in metastatic melanoma. The sICAM-1, sVCAM-1 and sIL-2R levels were all prognostic factors in univariate analysis, but only sIL-2R had prognostic power when adjusted for the metastatic site in multivariate analysis.

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