Soluble Triggering Receptor Expressed on Myeloid Cells-1 (sTREM-1) Detection in Cancer Patients: A Prognostic Marker For Lung Metastases from Solid Malignancies

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Abstract. *The aim of the study was to evaluate the serum soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) levels in breast, lung and colorectal cancer patients in correlation with clinical variables. Patients and Methods: A total of 59 patients with a median age of 64 years and histologically confirmed breast 14, colorectal 15 or lung cancer 30 were evaluated. Five patients with breast cancer, 7 patients with colorectal cancer and 8 patients with lung cancer had lung metastases. Blood was collected upon enrolment, centrifuged and the serum kept at –80˚C until assayed for sTREM-1. The estimation of sTREM-1 was performed by a crude enzyme immunoabsorbent assay. Results: High levels of sTREM-1 were observed in 50% of breast cancer, 33.3% of Small Cell Lung carcinoma (SCLC), 26.7% of colorectal cancer and 13.3% of Non Small Cell Lung Carcinoma (NSCLC) patients. sTREM-1 expression showed a correlation to the site of metastases. Higher concentrations were observed in the absence of lung metastases (p=0.019). Discussion: The novel mediator sTREM-1 may be a prognostic marker for the detection of lung metastases in metastatic and locally advanced solid tumors.*

The triggering receptor expressed on myeloid cells-1 (TREM-1) is a novel receptor highly expressed on the cell membrane of neutrophils and monocytes in the event of sepsis and septic shock (1, 2). Its soluble counterpart, designated as sTREM-1, has recently been described (3, 4). This molecule is highly elevated in the sera of patients with septic shock compared to patients with sepsis or severe sepsis, and it seems to behave as an anti-inflammatory mediator (5, 6). The same molecule has also been detected in high concentrations in the gastric fluid of patients with peptic ulcer disease; in whom it is positively correlated to gastritis (7).

The above data imply that sTREM-1 is a novel mediator, implicated in a variety of inflammatory processes ranging from gastritis to sepsis. It is possible that sTREM-1 may participate in other pathological conditions such as tumorigenesis and tumor progression.

The metastatic potential of several carcinomas has been the subject of many theories. The seed and soil theory initially proposed by Stephen Paget in 1899 (8) has gained widespread acceptance in the last twenty years. This theory suggests that cancer cells (the seeds) colonize only those secondary organs (the soil) that are able to support new tumor growth. In addition, the tumor microenvironment is characterized by the presence of tumor infiltrating inflammatory cells that contribute to cancer growth and spread (9). STREM-1 as a potent anti-inflammatory factor may play an inhibitory role in cancer-related inflammatory processes and thus contribute to restriction or inhibition of tumor spread.

This study was designed to enrol a population of patients, representative of common malignancies in order to define the level of sTREM-1 in the sera of patients with solid tumors and its possible correlation to the presence of distant metastases. The levels of sTREM-1 were estimated in the sera of patients with solid tumors.

Patients and Methods

A total of 59 patients were enrolled in the study between April 2004 and May 2006. All the patients gave written informed consent and approval was obtained from the ethics committee of Sotiria General Hospital. This study was conducted in accordance with the Helsinki declaration.

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Key Words: s-TREM-1, prognostic marker, lung metastasis.

Table I. *Baseline characteristics of 59 patients enrolled in the study.*

	Breast cancer	Colorectal cancer	Lung cancer
Number of patients	14	15	30
Gender (Male/Female)	0/14	10/5	25/5
Age in years (Mean±SD)	62.28±15.34	64.87±12.17	65.41±8.41
WBC, 10^3/μL (Mean±SD)	6.82±1.96	7.27±4.25	8.62±2.46

WBC: white blood cells.

Table II. *Clinical background of the patients enrolled in the study.*

a) LC: lobular carcinoma, DC: ductal carcinoma.

Breast cancer

	Histological type	
Stage (TNM)	LC	DC
IIb	1	1
IIIb	1	-
IV	6	5

b) NSCLC: non-small cell lung carcinoma, SCC: squamous cell carcinoma, AC: adenocarcinoma, SCLC: small cell lung carcinoma, LD: limited disease, ED: extensive disease.

Lung cancer

	NSCLC Histological type		
Stage	SCC	AC	Other
IIIa	1	1	
IIIb	1	1	
IV	3	3	5
	SCLC		
LD	2		
ED	13		

(c)

Colorectal carcinoma

Stage (Dukes')	Adenocarcinoma
B	2
C	3
D	10

Table III. *Site of metastasis of the patients included in the study.*

	Breast cancer	Colorectal cancer	Lung cancer
Site of metastases			
Lung	5	7	8
Liver	3	7	9
Lymph nodes	7	0	16
Bones	5	1	7

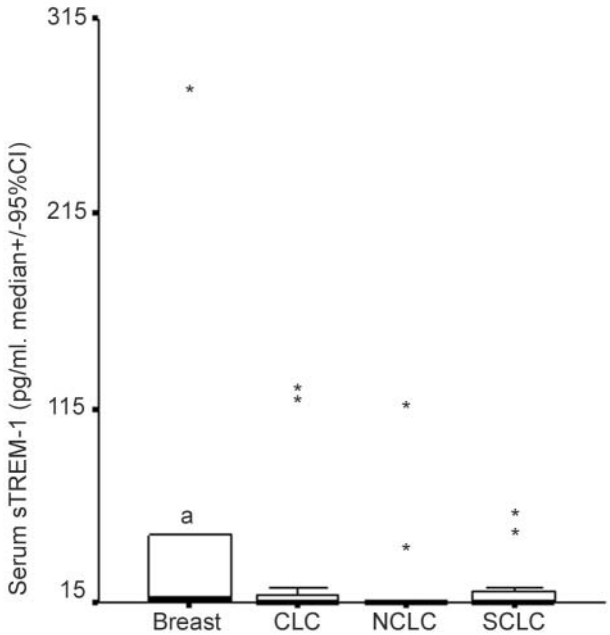

Figure 1. *Concentrations of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) in 59 patients with solid tumor malignancies. Asterisks denote outliers and circles extremes. Abbreviations: CLC, colorectal cancer; NCLC, non-small cell lung carcinoma; SCLC, small cell lung carcinoma, [a]p= 0.021 between breast cancer and NCLC.*

The patients were assessed by physical examination and history to ensure that eligibility criteria were met. Inclusion criteria included histologically confirmed breast, colorectal or lung cancer (cytological specimens obtained by brushing/washing or needle aspiration of a defined lesion were also acceptable) and age over 18 years. Exclusion criteria included unresolved toxicity from prior chemotherapy or incomplete healing from surgery, less than 20 days since radiotherapy, evidence of severe systemic disease or infection, white blood cells (WBC) less that 4,000/μl or over 11,000/μl and absolute neutrophil count less than 2,000/μl.

The median age of the patients was 64 (range 35-70) years. Thirty-five of the patients were male and 24 were female. Thirty patients with lung cancer [non-small cell lung cancer (NSCLC), n=15, small cell lung cancer (SCLC), n=15], 14 with breast cancer and 15 with colorectal adenocarcinoma were referred. Eleven out of the NSCLC, 13 out of the SCLC, 10 out of the colorectal cancer and 11 out of the breast cancer patients presented with distant metastasis at the time of diagnosis. Five patients with breast cancer, 7 patients with colorectal cancer and 8 patients with lung cancer had lung metastases. The presence of metastasis was ascertained by conventional imaging

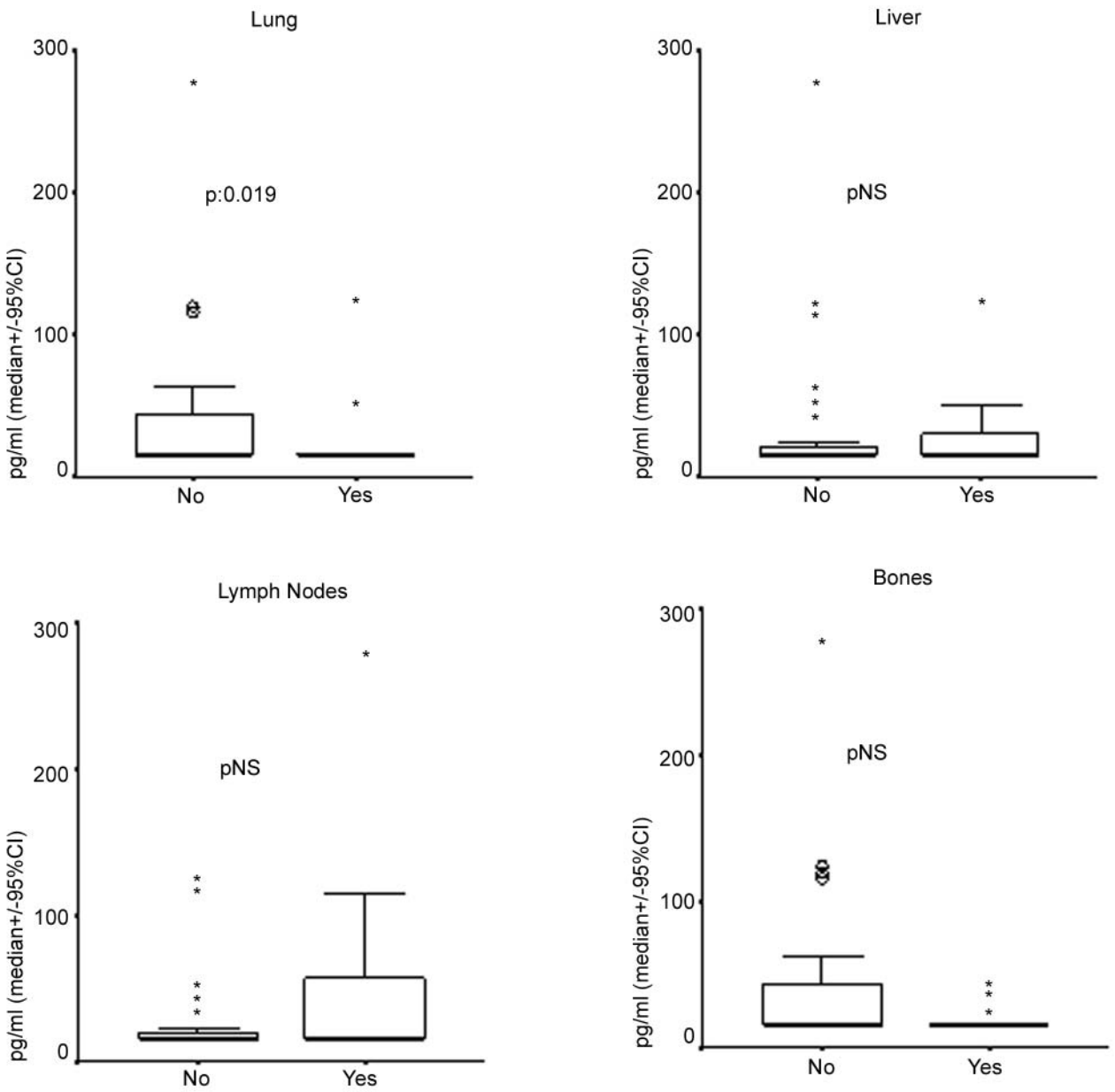

Figure 2. *Comparative concentrations of sTREM-1 in patients without and with the presence of metastasis to lung, liver, lymph nodes and bones. Asterisks denote outliers and circles extremes.*

methods (thorax, abdominal, brain computed tomographies and bone scintigraphy). The baseline characteristics of the patients and the site of distant metastasis are shown in Tables I, II and III.

Blood was collected upon enrolment, centrifuged, and the serum kept at minus 80˚C until assayed for sTREM-1. Estimation of sTREM-1 was performed by a crude enzyme immunoabsorbent assay. The capture antibody of sTREM-1 (R&D Inc., Minneapolis, USA) was diluted to 4000 ng/ml and distributed in a 96-well plate at a volume of 0.1ml per well. After overnight incubation at 250C, the wells were thoroughly washed with a 0.05% solution of Tween in phosphate buffered saline (PBS, Merck) (pH: 7.2-7.4). Then 0.1ml of standard concentrations of sTREM-1 (15.1-4000 pg/ml, R&D Inc) or serum was added to the wells. After incubation for two hours, wells were washed thrice and 0.1ml of 400 ng/ml dilution sTREM-1 detection antibody (R&D Inc) was added per well. The plate was then incubated for two hours, and attached antibodies were signalled by streptavidin. The concentrations of sTREM-1 in each well were estimated by the optical density detected at 450nm after addition of a 1:1 solution of H_2O_2 in tetramethylbenzidine as a substrate (R&D Inc.). All the determinations were performed in duplicate; the inter-day variation of the assay was 5.23%.

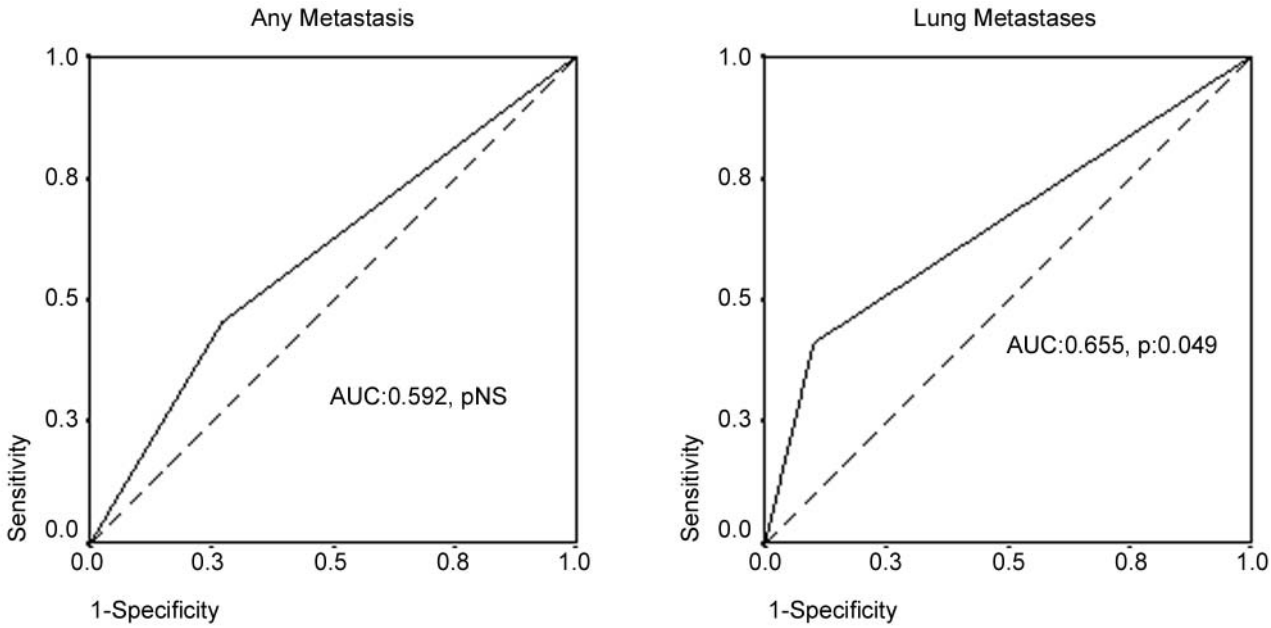

Figure 3. *ROC curves of the sensitivity and of the specificity of sTREM-1 to exclude the presence of any type of metastasis and of lung metastasis among 59 patients with solid tumor malignancies.*

Analyses were performed using SPSS software version 11.0 (SPSS, Inc., Chicago, IL, USA). All differences were considered positive if $p<0.05$.

Results

sTREM-1 concentrations in relation to the type of malignancy are shown in Figure 1. sTREM-1 was detected in the sera of seven patients with breast cancer (50%), four patients with colorectal cancer (26.7%), two patients with non-small lung carcinoma (13.3%), and five patients with small cell lung carcinoma (33.3%).

Differences in concentrations of sTREM-1 between patients with or without lung, liver, lymph nodes and bone metastases are shown in Figure 2. With the exception of the patients with lung metastases, no differences were found. Concentrations of sTREM-1 were higher among the patients without lung metastases compared to the patients with lung metastases ($p=0.019$).

ROC curves of the sensitivity and specificity of sTREM-1 as a marker for the absence of metastasis and lung metastasis in particular were performed (Figure 3). AUC of sTREM-1 to exclude the presence of all metastases was 0.592 (95%CI: 0.398-0.785, *p*NS). The sensitivity of sTREM-1 was 45.45%, specificity 72.91%, positive predictive value 85.36%, and negative predictive value 27.78%.

AUC of sTREM-1 to exclude the presence of lung metastases was 0.655 (95% CI: 0.514-0.796, $p=0.049$). The sensitivity of sTREM-1 for the absence of lung metastases was 41.03%, specificity 90.00%, positive predictive value 88.88%, and negative predictive value 43.90%.

Discussion

Since sTREM-1 is commonly found in the event of an infection, patients suffering from any underlying infection were excluded from the present study as were those with recent irradiation or surgical procedures that could have triggered an inflammation cascade.

sTREM-1 was detected in the serum of patients with breast, colorectal, non-small cell lung cancer and small cell lung cancer. The frequency of detection of sTREM-1 was higher in the patients with breast cancer compared to patients with the other malignancies. Furthermore, a connection seemed to exist between the absence of lung metastases and sTREM-1 detection. The patients without lung metastases had significantly higher concentrations of sTREM-1 than those with evidence of metastases. Similar differences were not detected for the other metastatic sites.

The ROC curves showed that sTREM-1 was characterized by considerable specificity and positive predictive values for the absence of any metastases. Moreover, sTREM-1 had statistically significant diagnostic impact with considerable specificity and positive predictive value for the detection of the absence of lung metastases.

The above findings are difficult to interpret since no data are available in the literature about the probable mechanism of biosynthesis and release of sTREM-1 in a patient with cancer. It could be hypothesized that sTREM-1 is secreted by similar mechanisms to those encountered in patients with systemic inflammatory states of non-infectious etiology such as vasculitis, gastritis and pancreatitis (7, 9).

It is known that metastasis depends on cross-talk between selected cancer cells and specific organ microenvironments. This means that the potential for tumor cells to metastasize depends on their interactions with the homeostatic factors that regulate tumor cell growth, survival, angiogenesis, invasion and metastasis. The tumor microenvironment often contains a number of migratory myeloid cells that play a pivotal role in tumor progression and metastasis (10). Monocytes are found in large numbers in the inflammatory infiltrates of tumors and it has been suggested that the presence of these cells may influence the tumor metastatic potential. Since sTREM-1 serves an anti-inflammatory role (6), it may be implicated in the interactions between cancer cells and homeostatic mechanisms.

In conclusion, the novel mediator, sTREM-1, was found in the serum of a large proportion of patients with breast cancer and its detection had considerable specificity and positive predictive value as a marker of the absence of lung metastases. Further research is required to elucidate its involvement in the biology of cancer and its significance as a surrogate marker.

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