NOWOTWORY Journal of Oncology • 2006 • volume 56
Number 5 • 513–516

Original papers

Evaluation of serum cytokine levels in the follow-up of patients with soft tissue sarcomas

Janina Kamińska[1], Maria Kowalska[1], Beata Kotowicz[1], Małgorzata Fuksiewicz[1], Piotr Rutkowski[2], Włodzimierz Ruka[2]

Introduction. The aim of this study was to evaluate the potential clinical use of circulating cytokine measurements in patients with soft tissue sarcomas during follow-up.
Patients and methods. The study comprised 69 patients with pathologically confirmed soft tissue sarcomas. All patients were treated according to institutional therapeutic protocols. In patients treated by surgery followed by adjuvant radio- or chemotherapy, cytokine levels were measured before treatment and 7-9 weeks after the treatment was completed. Out of all cytokines tested, IL-6, IL-8, sTNF RI and VEGF were chosen for monitoring of patients. Commercially available ELISA kits of R&D, Minneapolis, were used for serum cytokine assessment. Normal cytokine levels were assessed in 50 healthy blood donors. The Mann-Whitney and Wilcoxon tests were applied for statistical analyses.
Results. Serum IL-6 and IL-8 levels were significantly diminished after treatment in patients with disease remission, while in those with recurrent disease (local recurrences and/or metastases) no significant changes in serum cytokine concentrations was observed. Elevated pre-treatment serum IL-6 and sTNF RI concentrations were found more often in patients with soft tissue sarcomas with recurrent disease after treatment than in those who responded to treatment, but this difference has not reached statistical significance. Patients with recurrent disease presented significantly higher concentrations of serum IL-6, IL-8 and sTNF RI, as compared with patients with remissions.
Conclusion. In summary, out data suggest that changes in serum levels of the assessed cytokines may have a value of a follow-up marker, especially in patients with soft tissue sarcomas, where no standard tumour marker is known.

Key words: cytokines, soft tissue sarcoma, follow-up

Introduction

Cytokines comprise a heterogeneous group of proteins of high biological activity. These pleiotropic molecules are intercellular mediators that, via autocrine and paracrine way, control fundamental cell functions - proliferation, differentiation and migration, above all - and regulate immune reactions, inflammatory processes and physiological regeneration of tissues.

Excessive and uncontrolled expression of cytokine genes and their receptors in cancer cells as well as their increased synthesis in the stromal cells of the cancer site very often accompany neoplastic processes. Cytokines and their soluble receptors released to body fluids at sufficiently high concentrations may be useful markers for cancer diagnosis, prognosis, and monitoring of treatment response.

So far, most studies have focused on correlations between the levels of cytokines and their soluble receptors in blood serum and clinical and pathological characteristics of the disease as well as on the significance of serum cytokine concentrations as independent prognostic factors [1-5]. In our earlier studies, we have analysed the concentrations of an array of cytokines of various activities and their soluble receptors, to select those, which can prove to be most useful in oncological practice. Furthermore, for several cancer sites, analyses of cytokine and soluble cytokine receptor levels related to patient survival along with multivariate analyses revealed that some of these mediators might have a value of independent prognostic factors [1, 2, 6].

In only a few of the available papers the relationships between serum cytokine concentrations and the response to treatment was analysed [7-11].

In the study presented here, the potential use of selected serum cytokine measurements in the monitoring of treatment of patients with soft tissue sarcomas was assessed.

[1] Department of Tumour Markers
[2] Department of Soft Tissue/Bone Sarcomas
Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Warsaw, Poland

The study was approved by CCIO Bio-Ethics Committee according to Good Clinical Practice guidelines and all patients gave their informed consent.

Patients and methods

The study comprised 69 patients with soft tissue sarcomas. The characteristics of the patients is presented in Table I. All diagnoses were histologically confirmed. Patients with multiple cancers or with existing systemic inflammatory conditions were excluded from the study.

In all patients, serum cytokine levels were measured before and after treatment. Only patients treated with curative intent (surgical treatment with adjuvant radio or chemotherapy), with no signs of distant metastases at baseline, were included.

Patients were followed-up for the median time of over 3 years for the survivors.

Blood samples of 10 ml of were drawn before and after treatment. After clotting, the sera were collected by centrifugation, and stored at –70°C until assayed. The normal serum concentration ranges of cytokines and their soluble receptors were determined in 50 healthy volunteers [2].

Concentrations of cytokines and cytokine receptors were measured by enzyme-linked immunosorbent assay (ELISA) with the use of commercially available kits of R&D Systems, Minneapolis, USA. The tests were performed according to the manufacturer's instructions.

Statistical analyses

The Mann-Whitney test was used to compare independent samples, for dependent samples the Wilcoxon test was employed.

Table I. Patient characteristics

	Number of patients (%) n=69
Gender	
male	33 (48)
female	36 (52)
Age	18-84 (median 52 years)
Tumour pathology	
liposarcomas	20 (29.1)
malignant schwannomas	7 (10.1)
synovial sarcomas	8 (11.6)
MFH	11 (15.9)
leiomyosarcomas	7 (10.1)
others	16 (23.2)
Tumour size	
<10 cm	30 (43.5)
≥10 cm	39 (56.5)
Tumour grade	
G1	11 (15.9)
G2	24 (34.8)
G3	34 (49.3)

Results

Of the previously examined serum cytokines and soluble cytokine receptors (2), those that had been found to relate to various clinical and pathological features of soft tissue sarcoma or to have a prognostic value were selected to assess their use in monitoring the response to treatment. The following cytokines were studied: IL-6 and IL-8 possessing a prognostic value, and the pro-angiogenic VEGF as well as the soluble TNF receptor I (sTNF RI), found to correlate with the tumour size. These cytokines were also characterised by high diagnostic sensitivity.

Tables IIa and IIb present medians and ranges of serum cytokine concentrations and the percentages of patients with increased pre- and post-treatment cytokine levels, in patients with no recurrent disease in long-term follow-up (33 patients) and in patients with recurrent disease (36 patients). A statistically significant decrease in IL-6 ($p<0.003$) and IL-8 ($p<0.001$) concentrations was observed in patients with disease remission after treatment (Table IIa, Figure 1), but not in patients with recurrent disease (relapse/metastases, Table IIb).

Furthermore, the pattern of changes of the concentrations of cytokines in patients depending on their clinical course was analysed. Higher proportion patients

Table II. Serum cytokine levels in patients with soft tissue sarcomas and the results of treatment

a/ disease remission

Cytokines	Before treatment median	range pg/ml	Patients with elevated serum levels, %	After treatment median	range pg/ml	p value	Patients with elevated serum levels, %
IL-6	2.1	(0.7-135)	16/33 (48%)	1.1	(0.7-7.1)	0.003	6/32 (19%)
IL-8	14.2	(7.6-100.5)	19/33 (57%)	10.1	(10-28.7)	0.001	10/32 (31%)
VEGF	368	(50-1527)	19/32 (59%)	312	(61-1387)	NS	15/33 (45%)
sTNF RI	1359	(743-3633)	15/26 (58%)	1367	(600-3120)	NS	21/31 (68%)

NS = Not significant

b/ recurrent disease

Cytokines	Before treatment median	range pg/ml	Patients with elevated serum levels, %	After treatment median	range pg/ml	p value	Patients with elevated serum levels, %
IL-6	4.0	(0.7-129)	23/36 (64%)	3.2	(0.7-86.5)	NS	20/36 (55%)
IL-8	13.8	(10-149)	21/36 (58%)	13.3	(10-220)	NS	20/36 (55%)
VEGF	286	(30-2000)	17/34 (50%)	430	(29-2000)	NS	20/35 (57%)
sTNF RI	1288	(898-4186)	22/31 (71%)	1733	(744-5000)	NS	29/36 (80%)

NS = Not significant

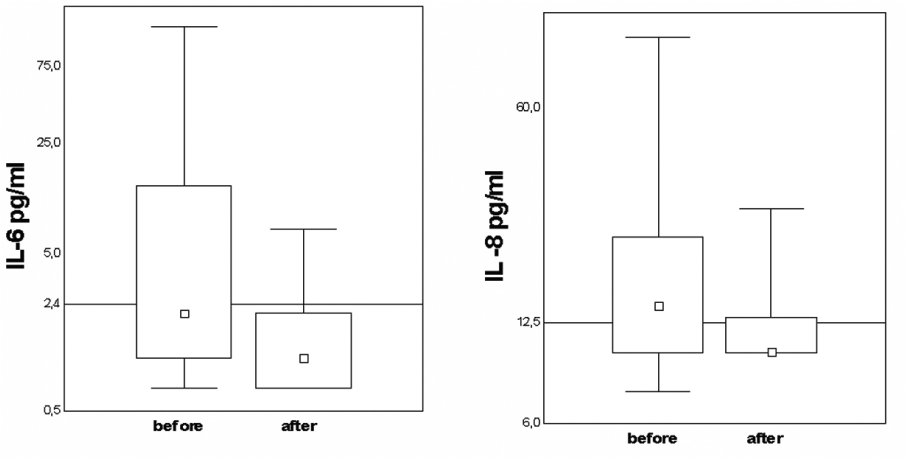

Figure 1. Serum IL-6 and IL-8 concentrations (ranges and medians) in patients before treatment and in patients after treatment with no recurrent disease

with recurrent disease than patients with disease remission presented elevated pre-treatment IL-6 and sTNF RI levels. However, these differences proved not statistically significant. On the other hand, significantly higher post-treatment concentrations of IL-6 ($p<0.001$), IL-8 ($p<0.05$) and sTNF RI ($p<0.02$) were observed in patients with recurrent disease than in those with remission (Figure 2).

Discussion

Determination of pre-treatment cytokine/soluble cytokine receptor levels in the blood serum may provide a useful prognostic tool and may enable a better stratification of patients for treatment. This applies especially to the cancers with no "classical" cancer markers known, such as soft tissue sarcomas. Therefore, it is necessary to search for the suitable markers which might be applied in widely understood diagnostics. Cytokines and their soluble receptors could serve this purpose. However, only few papers have dealt with this issue, in soft tissue sarcomas in particular [2, 11]. Much more frequently, cytokine levels have been related to specific clinicopathological features and prognosis than to treatment response [1, 3, 8, 9, 12-14]. To date, in patients with soft tissue sarcomas, the analysis of serum cytokine concentrations during

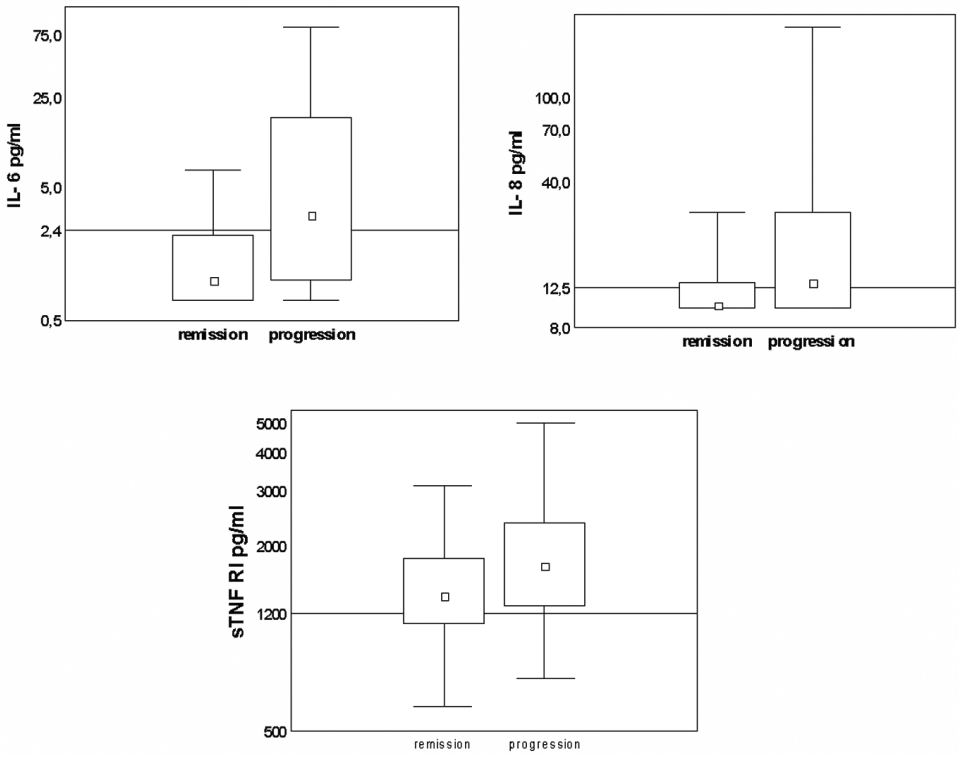

Figure 2. Serum IL-6 and IL-8 and sTNF RI concentrations (ranges and medians) in patients after treatment depending on clinical status

follow-up were the subject of only a limited number of studies, and the results obtained were inconsistent [2, 8, 9, 11].

Based on our previous data, the cytokines that play a role of important prognostic factors as well as those that correlate with various clinical and pathological features in patients with soft tissue sarcomas were selected for the current study, i.e. serum IL-6 and IL-8 shown to be independent prognostic factors, and VEGF and sTNF RI found to correlate with tumour size [2]. In this study, we have examined the potential use of the measurements of these cytokines in monitoring response to treatment. The subject of our analysis was not only the impact of treatment on cytokine concentrations. We have also studied the changes of serum cytokine levels depending on the response to treatment.

In patients with post-treatment disease remission, a statistically significant reduction in IL-6 and IL-8 concentrations was found. In contrast, in patients with recurrent disease (relapse/metastases), no statistically significant changes of serum IL-6 and IL-8 levels were observed. This may result from the fact that high percentage of patients with recurrent disease presented increased IL-6 levels already before treatment. On the other hand, when changes in cytokine concentrations were analysed as a function of the clinical status of patients, the proportion of patients with increased pre-treatment IL-6 and sTNF levels was higher among those with recurrent disease than in those with disease remission, but no statistical significance was reached. However, statistically significantly higher concentrations of prognostic cytokines, IL-6 and IL-8, and of sTNF RI were shown after treatment in patients with recurrent disease as compared with those with disease remission.

In the previously investigated cancer sites, we have shown markedly higher concentrations of sTNF RI, but not of TNFα [1, 2, 6]. The TNF RI (55 kD) is a surface receptor, expressed by basically all cells, but first of all by epithelial cells. Both TNF RI and RII, but TNF RI in particular, contains the so-called death domains that trigger the apoptotic process following the receptor-ligand binding. Therefore, the release of TNF receptors by cancer cells is considered a likely element of cellular defence against apoptosis, though no strong evidence confirms this idea [15].

Summary. In patients with soft tissue sarcomas, a change in the serum cytokine concentrations during follow-up is related to disease recurrence. In particular, the measurements of serum IL-6, IL-8 and sTNF RI levels may be useful in monitoring of treatment response in patients with soft tissue sarcomas, where no standard cancer marker is known.

Janina Kamińska MD, PhD
Department of Tumour Markers
Maria Sklodowska-Curie Memorial Cancer Centre
and Institute of Oncology
Roentgena 5, 02-781 Warsaw, Poland

References

1. Kaminska J, Nowacki MP, Kowalska M et al. Clinical significance of serum cytokine measurements in untreated colorectal cancer patients: soluble tumor necrosis factor receptor type I – an independent prognostic factor. *Tumor Biology* 2005; 26; 186-94.
2. Rutkowski P, Kamińska J, Kowalska M et al. Cytokine serum levels in soft tissue sarcoma patients: Correlations with clinico-pathological features and prognosis. *Int J Cancer* 2002; 100; 463-71.
3. Belluco C, Nitti D, Frantz M et al. Interleukin-6 blood level is assotiated with circulating carcinobryonic antigen and prognosis in patients with colorectal cancer. *Ann Surg Oncol* 2000; 7: 133-8.
4. Martin F, Santolaria F, Batista N et al. Cytokine levels (IL-6 and INF-γ), acute phase response and nutritional status as prognostic factors in lung cancer. *Cytokine* 1999; 11: 80-6.
5. Punnonen R, Teisala K, Kuoppala T et al. Cytokine production profiles in the peritoneal fluids of patients with malignant or benign gynecologic tumors. *Cancer* 1998; 83: 788-96.
6. Rutkowski P, Kamińska J, Kowalska M et al. Cytokine and cytokine receptor serum levels in adult bone sarcoma patients: correlations with local tumor extent and prognosis. *J Surg Oncol* 2003; 1-9
7. Giacomelli L, Gianni W, Belfiore C et al. Persistence of epidermal growth factor receptor and interleukin 10 in blood of colorectal cancer patients after surgery identifies patients with high to relapse. *Clin Cancer Res* 2003; 9: 2678-82.
8. Yoon SS, Segal NH, Olshen AB et al. Circulating angiogenic factor levels correlate with extent of disease and risk of recurrence in patients with soft tissue sarcoma. *Ann Oncol* 2004; 15: 1261-6.
9. Yudoh K, Kanamori M, Ohmori K et al. Concentration of vascular endothelial growth factor in the tumour tissue as a prognostic factor of soft tissue sarcomas. *Br J Cancer* 2001; 84: 1610-5.
10. Chin KF, Greenman J, Reusch Pet al. Vascular endothelial growth factor and soluble Tie-2 receptor in colorectal cancer: associations with disease recurrence. *Eur J Surg Oncol* 2003; 29: 497-505.
11. Graeven U, Andre N, Achilles E et al. Serum levels of vascular endothelial growth factor and basic fibroblast growth factor in patients with soft-tissue sarcoma. *J Cancer Res Clin Oncol* 1999; 125: 577-81.
12. De Vita F, Romano C, Orditura M et al. Interleukin-6 serum level correlates with survival in advanced gastrointerstinal cancer patients but is not an andependent prognostic indicator. *J Interferon & Cytokine Res* 2001; 21: 45-52.
13. Iyoda A., Kenzo H., Masayuki B. et al. Expression of vascular endothelial growth factor in thoracic sarcomas. *Ann Thorac Surg* 2001; 71: 1625-9.
14. Mroczko B, Szmitkowski M. Hematopoietic cytokines as tumor markers. *Clin Chem Lab Med* 2004; 42: 1347-54.
15. Aderka D. The potential biological and clinical significance of the soluble tumor necrosis factor receptors. *Cytokine & Growth Factor Rev* 1996; 7: 231-40.

Paper received: 7 February 2006
Accepted: 27 March 2006