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Serum Soluble Tumour Necrosis Factor Receptor 55 is Increased in Patients with Haematological Neoplasias and is Associated with Immune Activation and Weight Loss

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Enhanced concentrations of soluble forms of the receptor for tumour necrosis factor (TNF)-α have been detected in the serum of cancer patients. We determined serum concentrations of soluble TNF receptor p55 (sTNF-R55) in patients with haematological neoplasias, 50 patients suffering from non-Hodgkin's lymphoma (n = 35), Hodgkin's disease (n = 10) and multiple myeloma (n = 5). Compared with healthy controls and with patients with potential thyroid disease, significantly elevated concentrations of sTNF-R55 were found (mean ± standard error: 2.68 ± 0.22 vs. 1.23 ± 0.21 ng/ml, $P < 0.0001$ and 2.18 ± 0.32 ng/ml, $P = 0.03$). Likewise, neopterin concentrations were raised (19.6 ± 3.66 vs. 5.3 ± 0.25 nmol/l in controls, $P < 0.0001$). We found a significant correlation between sTNF-R55 and neopterin concentrations (Rs = 0.544, $P < 0.001$). Patients with weight loss showed higher sTNF-R55 concentrations than patients with stable weight. Our results confirm the relevance of sTNF-R55 concentrations in serum of patients with cancer.

Keywords: cachexia, haematological neoplasias, neopterin, sTNF-R55, tumour necrosis factor-α, weight loss.
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INTRODUCTION

WITHIN THE past few decades cytokines have been of increasing interest in the field of malignant disorders [1, 2]. Besides other cytokines like interferon-γ (IFN-γ), interleukin (IL)-1 and IL-2, tumour necrosis factor (TNF)-α appears to play an important role in the development of cachexia and mediation of weight loss in patients with malignant diseases [3–8]. However, the role of cytokines in patients is not fully elucidated and the information often remains incomplete, because many factors are active within very restricted environments. Also, the determination of cytokine levels in the circulation still raises problems, since endogenously released antibodies to cytokines, inhibitors and soluble forms of cytokine receptors may interfere with the determination of cytokine levels [9].

Measurement of soluble TNF receptors (sTNF-Rs) allows some insight into TNF biology and bears some advantage compared to direct quantification of TNF since sTNF-Rs are very stable and can also be determined in stored sera. Recently, elevated concentrations of sTNF-Rs were described in cancer patients compared to healthy controls [10]. Both of the two distinct forms of sTNF-Rs, sTNF-R55 as well as sTNF-R75, were found to be raised in cancer patients.

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The aim of the present study was to determine sTNF-R55 concentrations in the serum of patients with haematological neoplasias and to compare the values with signs of endogenous immune activation, measured by neopterin concentrations, and with clinical parameters. In addition, concentrations of sTNF-R55 were compared to weight loss in patients.

PATIENTS AND METHODS

Cases

50 patients (31 males, 19 females) were included in the study. All patients suffered from haematological malignancies: non-Hodgkin's lymphoma (NHL, n = 35; 18 low-grade, 14 high-grade, 2 T-cell lymphoma, 1 not further classified), Hodgkin's disease (HD, n = 10), multiple myeloma (MM, n = 5). 17 out of 50 (34%) patients were evaluated before the start of treatment, 19 patients (38%) were under therapy and 14 (28%) were without treatment because of remission or stable disease. Staging of patients with NHL and HD was performed according to the Ann Arbor classification [11], patients with MM were staged according to the classification of Durie and Salmon [12].

Body weight was examined and compared to previous values from the medical records. The extent of weight loss within the last 6 months was judged as percentage of total body weight as well as in absolute values (kg).

Controls

Thirty sera from patients with potential disease of the thyroid and 23 sera of blood donors (male and female) served as controls with respect to sTNF-R55.

Analytical procedures

Blood obtained by venepuncture was allowed to clot at room temperature for 1 h. Serum was stored at −20°C before measurement of sTNF-R55 and neopterin.

Concentrations of sTNF-R55 were measured with enzyme-linked immunological and biological binding assay (ELIBA) using an automated form (Cobas-Core; Hoffmann–LaRoche, Basel, Switzerland) of the test system described [13]. In brief, plastic balls sensitised with monoclonal (mouse) antibodies directed against sTNF-R55 (Clone HTR-20) were reacted in a one-step reaction with either TNF-R55 contained in the patient's sera or with standard solutions of recombinant sTNF-55 and the conjugate of human recombinant TNF-α–horseradish peroxidase. A mixture of tetra-methyl benzidine and hydrogen peroxide served as an indicator. The developed colour is directly proportional to the sTNF-R55 concentration in the sample or standard. Clone HTR-20 is highly specific for human and recombinant TNF-R55 and does not crossreact with lymphokines and cytokines, such as interferons, interleukins or growth factors.

Serum neopterin concentrations were determined by radioimmunoassay (IMMUtest, Henning-Berlin, Germany) as described previously [14]. The 95th percentile of serum neopterin concentrations in healthy controls is ≤ 8.7 nmol/l.

Statistical methods

For the evaluation of differences between various groups of patients we used the Wilcoxon–Mann–Whitney U-test. Incidences were compared by the χ^2 test. For the definition of strength and significance of correlations Spearman's rank correlation was used. P values below 0.05 were considered to indicate statistical significance.

RESULTS

We found significantly elevated concentrations of sTNF-R55 in the serum of patients with haematological neoplasias compared with the group of patients with potential thyroid disease (U = 2.12, P = 0.03) and with healthy blood donors (U = 4.44, $P < 0.0001$). The highest concentrations of sTNF-R55 were found in patients with NHL, whereas patients with HD mostly showed normal values (Table 1). Neopterin concentrations were found to be raised in the majority of patients. In patients there existed a highly significant correlation between the concentrations of sTNF-R55 and neopterin (Rs = 0.544, $P < 0.001$; Fig. 1). There was no difference of sTNF-R55 concentrations between subtypes of NHL, namely patients with low grade ($\bar{x}$ ± S.D. 2.62 ± 1.70 ng/ml) and high grade (2.69 ± 1.85; P = not significant).

Weight loss within the last 6 months was found in 18 patients (36%). Average loss of weight was 9% (5.9 kg), the range was between 3 and 14 kg (3–25%). 1 out of 10 patients with HD had weight loss, whereas this symptom was found in 15/35 (43%) patients with NHL. In myeloma patients the percentage was 40% (2/5).

Table 1. sTNF-R55 and neopterin concentrations (mean ± S.E.) in patients with haematological neoplasias

	n	sTNF-R55 (ng/ml)	Neopterin (nmol/l)
Patients	50	2.68 ± 0.22	19.6 ± 3.66
NHL	35	2.74 ± 0.28	23.5 ± 5.02
HD	10	2.44 ± 0.53	6.9 ± 1.04
MM	5	2.64 ± 0.15	17.9 ± 6.17
Controls			
Thyroid patients	30*	2.18 ± 0.32	Not done
Blood donors	23*	1.23 ± 0.21	5.3 ± 0.25 [14]

*Controls for sTNF-R55 measurements. NHL, non-Hodgkin's lymphoma; HD, Hodgkin's disease; MM, malignant melanoma.

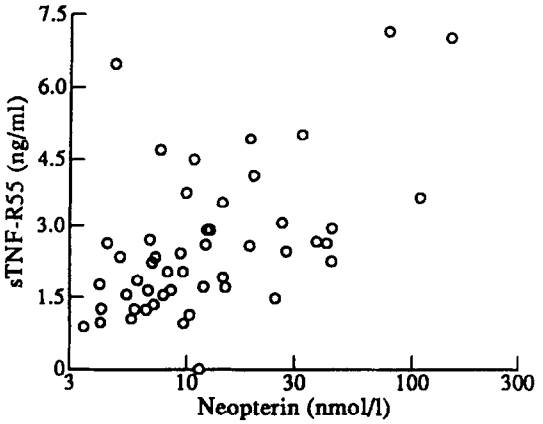

Fig. 1. Correlation between sTNF-R55 and neopterin concentrations in patients with haematological neoplasias (Rs = 0.544, $P < 0.001$, note logarithmic scale of neopterin data).

In 9/17 (53%) patients investigated before the start of therapy weight loss was found. In patients with therapy the fraction was 6/19 (32%), whereas only 3 out of 14 patients (21%) in remission or stable disease showed decrease in body weight. The average loss was higher in pretherapeutic and treated patients than in patients without therapy (9.8 vs. 6.0%, $P < 0.05$).

Patients with weight loss showed significantly higher values of sTNF-R55 than patients with stable body weight (U = 2.8, P = 0.005). These results are graphically depicted in Fig. 2. As with neopterin, we found a significant correlation between the sTNF-R55 concentrations and the degree of weight loss, expressed both as percentage body weight (Rs = 0.379, $P < 0.01$) and in absolute numbers (kg) (Rs = 0.383, $P < 0.01$) (Fig. 3). Among the subgroup of patients with NHL, similar results were obtained: patients with weight loss had higher sTNF-R55 ($\bar{x}$ ± S.D. 3.62 ± 2.03 ng/ml) and neopterin (36.4 ± 37.1 nmol/l) concentrations than patients with stable

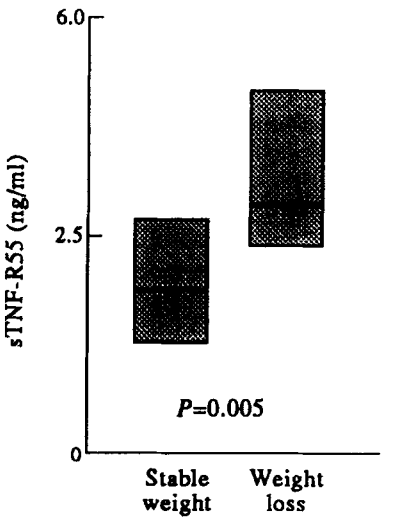

Fig. 2. Concentrations of sTNF-R55 in patients with stable body weight compared to patients with more than 5% weight loss (medians and interquartile ranges are shown).

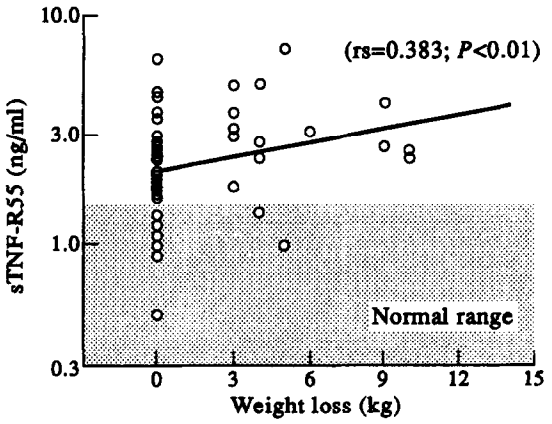

Fig. 3. Correlation between concentrations of sTNF-R55 and weight loss in patients with haematological neoplasias (note that concentrations of sTNFR-55 are shown in logarithmic scale, the correlation coefficient shown results from Spearman rank correlation analysis).

weight (sTNF-R55 2.18 ± 1.19, $P < 0.05$; neopterin 13.0 ± 20.2, $P < 0.001$).

DISCUSSION

Sera of patients with haematological neoplasias have elevated levels of sTNF-R55. This result agrees with previous data reporting increased sTNF-R concentrations in patients with solid tumours [10]. A strong correlation exists between signs of immune activation as detected by increased neopterin levels and elevated sTNF-R55 concentrations. This correlation favours the view that sTNF-R55 is produced by cells of the immune system. It also favours the view that, as for neopterin formation [17], activated macrophages are the major source for release of sTNF-R55. However, a role of other lymphoid cells to contribute to increased sTNF-R55 in the patients cannot be ruled out. Similar to the secretion of neopterin, it appears that IFN-γ also plays a role in inducing the formation and the shedding of sTNF-R55 by macrophages. Recently it has been demonstrated *in vitro* that IFN-γ is able to enhance expression of TNF receptors on cell surfaces and to increase shedding of its soluble form [15].

In an earlier study we found an association between increased neopterin levels and weight loss in patients with haematological neoplasias [16], indicating a role of a stimulated cellular immune system in the development of cachexia. Increased concentrations of neopterin, a pteridine derivative which is secreted by macrophages upon stimulation by IFN-γ, indicate the activation of the cell-mediated immune system in humans [17]. Increased neopterin concentrations are frequently detected in patients with haematological neoplasias [16, 18] and simultaneously correlate well with the degree of anaemia [19]. In addition, raised neopterin concentrations were shown to be associated with a worse prognosis in patients with malignant lymphomas and with human immunodeficiency virus type 1 (HIV-1) infection [18, 20]. Neopterin is a stable molecule easily detected in the circulation. It signals the presence of IFN-γ in the serum [17, 21]. In patients with HIV-1 infection a similar association between increased sTNF-R55 and neopterin was found [22]. This is of particular interest because an association between higher neopterin levels and the development of cachexia was also observed in this group of patients.

On the one hand, TNF-Rs are able to abrogate some functions of TNF [10]. On the other hand, they may have the function of stabilising circulating TNF [23]. The association of serum concentrations with weight loss found in our patients confirms the clinical relevance of sTNF-R55. We assume that enhanced concentrations of sTNF-R55 in the circulation are related to increased surface expression of TNF receptors. Upregulation of TNF receptors on cell surfaces may increase their sensitivity to respond to endogenously released TNF in an autocrine manner. As neopterin secretion by macrophages is stimulated by IFN-γ, the data suggest that the latter cytokine may enhance responsiveness of cells in general and tumour cells in particular to TNF. IFN-γ and TNF-α may, therefore, play an essential role in the pathogenesis of tumour cachexia. It is even possible that neopterin itself takes part in the pathogenesis of cachexia. Recently, neopterin was shown to increase intracellular calcium by activation of a calcium channel *in vitro* [24] and to amplify radical-mediated cytotoxicity *in vitro* [25]. Further investigations are needed to clarify this point.

In conclusion, our data show that sTNF-Rs can easily be detected even in stored sera. Concentrations of sTNF-R55 correlate with signs of immune activation reflected by neopterin levels and are associated with the development of cachexia. Changes of sTNF-R55 concentrations present another part of the puzzling network of cytokines involved in the complex pathogenesis of malignant diseases.

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Information for Cancer Patients Entering a Clinical Trial—an Evaluation of an Information Strategy

Anders Bonde Jensen, Biddy Madsen, Poula Andersen and Carsten Rose

Informing patients before the start of antineoplastic treatment is important due to the anxiety and uncertainty felt by the patients and the legal aspects of trials. 34 women were interviewed 3 months after receiving information. Results show that the information was well remembered, patients were glad to bring a relative, two consultations with time for deliberation were well-received and that patients viewed written information as an important reinforcement. Overall, information provided was positively evaluated. Detailed information allowed patients to understand and participate in treatment decisions, thereby reducing their pretherapy anxiety. These results support expansion of the structured information programme to include all patients about to begin long-term cancer therapy.
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INTRODUCTION

INFORMING PATIENTS before they begin a complex, antineoplastic treatment is an increasingly important aspect of patient care. Today, cancer treatment often takes place in the context of a clinical trial, so the primary information must serve several purposes.

Firstly, the information should reduce patients' feelings of anxiety and uncertainty towards treatment and disease. There are many myths about cancer and the admittedly serious side-effects of treatment with chemo- or radiotherapy, which amplify the anxiety about treatment to almost unbearable proportions. To reduce these fears there is a need for understandable, factual information about the disease and its treatment [1].

Secondly, the information should provide patients with all details about the clinical trial essential for them to make an autonomous decision regarding participation. The Helsinki II Declaration provides the clinician with guidelines on the information patients should receive before consenting to enter a trial [2]. These ethical rules are the result of a process which started in the early 1930s, when the first regulations were passed addressing the rights of volunteers in clinical trials [3, 4], and which were later detailed in the Helsinki I and II Declarations [5, 6]. Accompanying this development has been a growing public demand for more information about results from diagnostic investigations, standard treatments, and especially participation in clinical trials, as well as an increased respect for the autonomy of the individual patient.

If this required consent is to be truly "informed", factual information must be given in such a way as to allow the patient to transform the information into knowledge making him or her capable of self-determination, and thereby competent to give a valid informed consent. In a randomised study, comparing total disclosure and individual approach, it was found that total disclosure gave a better understanding of treatment and trial conditions, but also a higher degree of anxiety [7].

In a previous study at our Department of Oncology, we found several factors which reduced the likelihood of obtaining valid informed consent: (a) an unstructured manner of giving the information used by the doctor, (b) lack of time to consider the information before giving consent, and (c) anxiety and nervousness of the patients. Based upon this analysis [8, 9], we

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