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Serum TNF-α Level in the Neoplasm Patients Qualified for Surgical Treatment

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Abstract. The aim of the study was to analyze serum tumor necrosis factor alpha (TNF-α) levels in patients with different neoplasm types qualified for surgical treatment and to evaluate their possible correlations with circulating immune complexes (CIC), IgG, IgM, and the complement (C) compounds: C1 inhibitor (C1i), C3c and C4 levels. Studies were performed in sera from 30 neoplasm patients before surgical treatment and in 10 persons from a control group (CG) with no malignancy. Serum TNF-α levels were measured with the Cytogen ELISA kit. Average TNF-α levels measured in neoplastic patient groups qualified for surgical treatment were not significantly different from the average TNF-α level in the CG group.

Key words: neoplasm(s); TNF-α; serum; surgery.

Introduction

Since the first report on tumor necrosis factor alpha (TNF-α) in 1975[4] as an endotoxin-induced serum factor that causes necrosis of tumors, significant progress has been made in studies on the role of this cytokine in health and disease. TNF-α is now known to be produced by many types of cells in the absence of infection and inflammation[3, 20, 22, 23]. Consequently, endogenous TNF-α has been postulated to serve as a modulator of normal tissue homeostasis[20, 22, 23]. In some non-malignant diseases, e.g. in cardiac infarction[17], chronic heart failure[24] or in pneumonia[12], serum TNF-α levels are several times higher than those of both healthy people as well as of neoplastic patients.

The mechanisms of growth inhibition and regression of tumors by TNF-α may be due to both the direct effect of TNF-α on the tumor cells and its indirect effect on the tumor vasculature and/or the host immune system[5]. WATANABE et al.[21] have shown that cancer cell destruction with TNF-α can proceed from injury of tumor blood vessels. However, tumor vasculature is

Abbreviations used: C – complement, C1i – complement 1 inhibitor, CG – control group, BC – breast or ovary cancer group, DT – digestive tract cancer group, M – melanoma patients group, ON – other neoplasms, CIC – circulating immune complexes, TNF-α – tumor necrosis factor alpha, sTNF-Rs – soluble TNF-α receptors, r – coefficient of correlation, R^2 – coefficient of determination.

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known to be structurally different from that of normal blood vessels[2]: endothelial cells of normal vessels are far less susceptible to TNF-α than are those of newly formed tumor vasculature[21].

TNF-α may activate macrophages to produce factors that potentiate TNF-α cytotoxic action and can affect other cells (neutrophils, lymphocytes), either by influencing their adhesion to endothelium or by immediate changes of their surface[5]. These findings suggest that the mechanism of action of TNF-α *in vivo* may involve the activation of the host immune system and/or direct action on the local vasculature of the tumors[5].

Still, there are several pitfalls in the TNF-α – tumor growth stimulation/inhibition reasoning. For example, no correlation between TNF-α and tumor growth stages has been found in bladder cancers[18]. Another report has revealed that, paradoxically, transplantation of neoplasms into mice after a previous administration of TNF-α increased the number of metastases in the lungs, which correlated with the TNF-α doses[15].

The present study was undertaken in the belief that one of the necessary conditions for neoplasm development is an inefficiency of the defensive mechanisms in neoplastic cells' removal. This insufficiency may be a result of both the specific neoplastic cell characteristics and specific or general immunological deficiencies. It is undoubtful that several of the organism's functions and defence mechanisms change depending on the neoplasm type and its stage. It can be assumed that qualification for surgical treatment allows the selection of a group of patients in whom a final decline in health and final immunological decay have not been approached.

The aim of the study was to analyze serum TNF-α levels in different neoplasm types qualified for surgical treatment and to evaluate their possible correlations with selected concomitantly studied humoral index levels.

Materials and Methods

The studies were performed with sera from 30 neoplasm patients before surgical treatment and from 10 persons of a control group (CG) with no malignancy. The oncologic patients were divided into 4 subgroups, depending on the affected organ: breast or ovary cancer (BC) – 13 patients, digestive tract (DT: stomach or colorectal cancers) – 11 patients, melanoma cutis (M) – 4 patients and other neoplasms (ON: lymphoma and liposarcoma) – 2 patients, as described earlier[9]. All these neoplasms were of stage I or II. Preoperative estimations were confirmed during operation.

The persons with no malignancy made the control group (CG); these were volunteers recruited from outpatients complaining of mild heart symptoms.

Serum TNF-α levels were measured with the Cytogen ELISA kit. The assay is based on a sandwich-technique, with a monoclonal antibody against TNF-α as the capture antibody and a second biotinylated monoclonal antibody for detection. This second antibody reacts with streptavidin-peroxidase. The bound enzymatic activity is determined by addition of a chro-

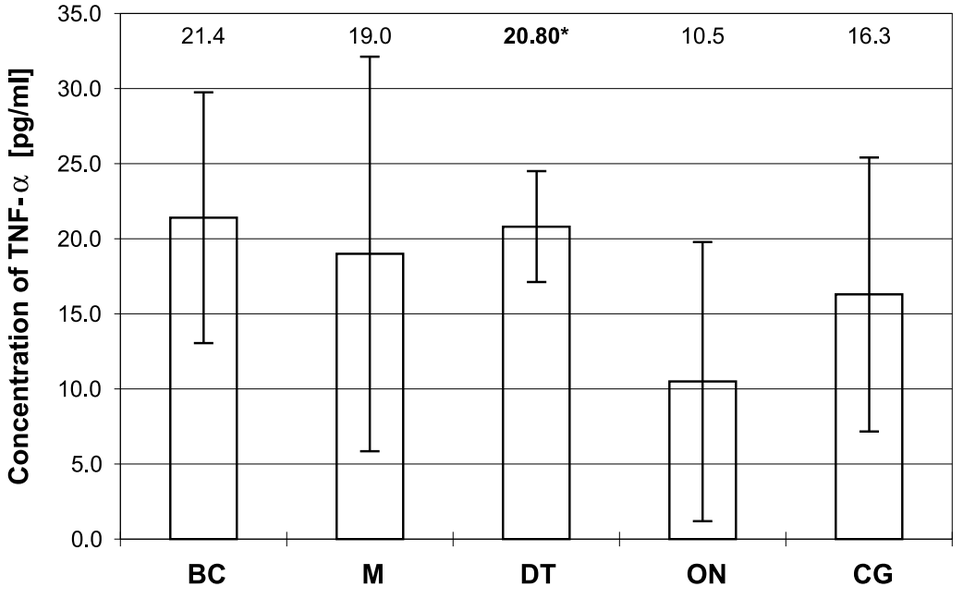

Fig. 1. Average TNF-α concentrations in the studied groups (no significant differences between the groups shown by the Student's *t*-test). * The statistically significant difference as compared with CG ($p<0.029$) and to M ($p<0.01$) by means of the F-Fisher-Snedecore's test

mogenic substrate (TMB). The obtained TNF-α concentrations were used for calculating average values within the groups and for assessing correlations between TNF-α levels and the humoral immunity indeces' levels studied earlier by us[9], e.g. circulating immune complexes (CIC), IgG, IgM and the complement compounds: C1 inhibitor (C1i), C3c and C4. The methods of analysis of these factors' are described in detail in the above-mentioned paper[9]. Having screened the results with Q-Dixon's test, average values and standard deviations were calculated and plotted in a histogram (Fig. 1). Variances of TNF-α concentrations were compared by means of F-Fisher-Snedecore's test. In case of statistical significance of the difference between the CG and a patient group detected with this test, an asterisk is used to indicate that fact in the figure.

For comparisons of average values, the non-paired Student's *t*-test was used and the detected statistical significance of the differences is shown with "p <" or "p =" the appropriate value, while in case of insignificance of the differences, "p > 0.05" or no information on p is indicated in the appropriate place.

Results

The average serum TNF-α concentrations in the neoplasm patient groups were not significantly different (according to the Student's *t*-test) from the average value for the CG (Fig. 1).

The F-test allowed detecting differences of variances calculated for the DT group and the CG ($p < 0.029$), indicating differences in the TNF-α concentration distribution among these two groups. The parameter for the DT group was also significantly different from the one calculated for melanoma (M) patients ($p < 0.01$). Concomitant assessments of CIC, IgG, IgM and complement compounds (C1i, C3c and C4) allowed analysis of the correlations between these and the TNF-α concentrations in the tested groups (Table 1). The following statistically significant correlations were found: a negative correlation between TNF-α and IgG levels in the CG ($r = –0.692$; $p < 0.05$) (Fig. 2) and a positive correlation between TNF-α and C3c in the M group ($r = 0.956$; $p < 0.05$) (Fig. 3). No statistically significant correlations were found in the other groups between TNF-α and the other protein levels studied (Table 1). Additionally, some of the high but insignificant correlations are shown in Fig. 4.

Discussion

The considerable individual differences observed in serum TNF-α levels among persons from both the CG and the neoplasm patient groups studied (together with a lack of significant differences between the average values calculated for the groups) do not exclude the possibility of a dynamic influence of TNF-α on humoral immunity phenomena. The above suggestion seems to be confirmed, in a way, by the detected diversity in the correlations between serum TNF-α levels and some humoral immunity indices in the CG and neoplasm patient groups studied.

The negative correlation observed by us between TNF-α and IgG levels in the CG ($r = –0.692$; $p < 0.05$) seems to agree nicely with the KOWALCZYK et al. findings[13]. Interestingly, these authors reported that hypo-

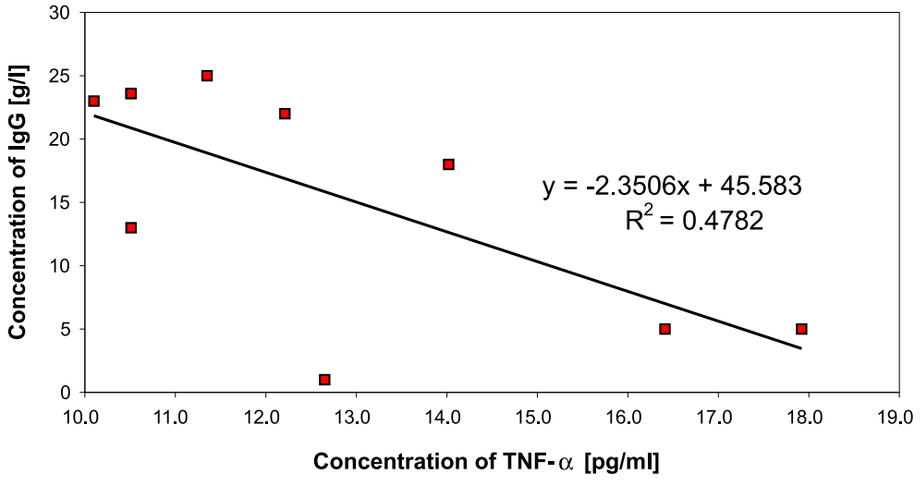

Fig. 2. Regression analysis for results of negative correlation between TNF-α and IgG levels in CG sera ($r = –0.692$, $p < 0.05$). The coefficient of determination (R^2) and the regression equation shown in the figure

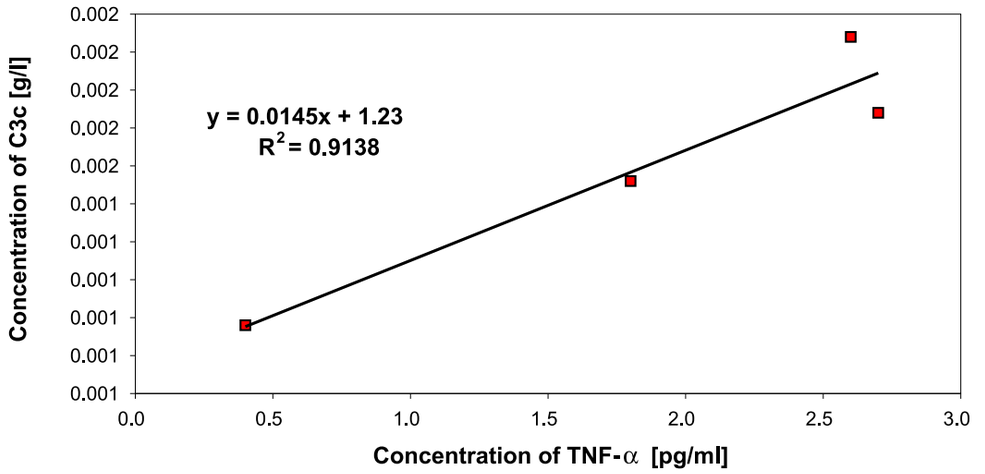

Fig. 3. Regression analysis for results of TNF-α and C3c concentrations in M sera (coefficient of correlation r = 0.956, $p < 0.05$; the coefficient of determination (R^2) and the regression equation shown in the figure)

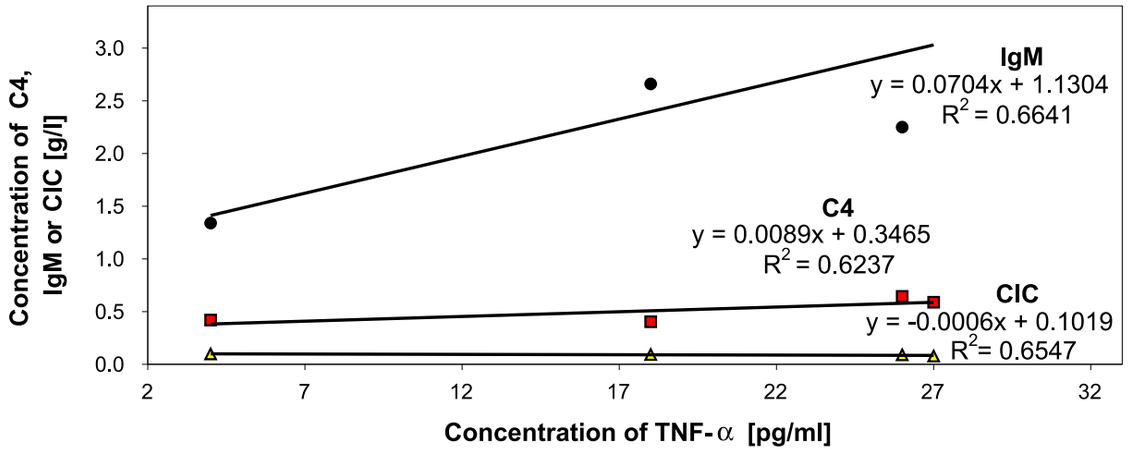

Fig. 4. Results of TNF-α, C4 and IgM analysis in M sera. Correlations: TNF-α/C4 (r = 0.790), TNF-α/IgM (r = 0.815) and TNF-α/CIC (r = –0.809). The coefficient of determination (R^2) values and the regression equations shown in the figure

Table 1. Correlations between serum TNF-α and other immunity indices studied coefficient of correlation (r) values, significant values have been typed in bold

Parameter	Group			
	CG	BC	DT	M
Age	0.108	0.200	0.175	–0.200
CIC	–0.448	0.200	0.199	–0.809
IgG	**–0.692**	0.130	–0.199	–0.042
IgM	–0.123	0.120	–0.575	0.815
C1i	0.599	–0.410	0.404	0.471
C3c	–0.278	0.070	0.030	**0.956**
C4	0.397	–0.293	–0.263	0.790

gammaglobulinaemia prompted a transient increase in TNF-α production in cultured mitogen-stimulated lymphocytes[13]. Thus, it is possible that the observed by us negative correlation between TNF-α and IgG levels in the CG reflects the presence of such a dependency or even a feedback. Why can we not find this dependency in the cancer patient groups? What mechanisms are responsible for the destruction of these interrelationships between TNF-α and IgG levels? It cannot be excluded that soluble TNF-α receptors (STNF-Rs), playing a role as modulators of the biological function of TNF-α in an agonist/antagonist pattern, can contribute to these effects, since STNF-Rs can mediate host response and determine the course and outcome of a disease by interacting with TNF-α and competing with cell surface receptors[8], which can cause a decrease in cell surface availability for TNF-α. According to another paper, STNF-Rs may protect cells from cytotoxic/cytostatic effects of locally released TNF-α[14].

We found the significant positive correlation between TNF-α and C3c in the M group ($r = 0.956$; $p < 0.05$) (Fig. 3) mentioned above. According to literature data, some *in vitro* studies demonstrated that TNF-α alone was able to stimulate an increase in C3 production, for example in gastric cancer-derived cell lines[11] and in human intestinal epithelial cells[1]. Additionally, TNF-α, acting together with other cytokines, induced C3 synthesis in fibroblasts[10] and intestinal epithelial cells[1]. Isolated limb perfusions with recombinant TNF-α in cancer patients resulted in C3 and C4 serum level increases[19]. The question arises as to why TNF-α in our studies seemed to stimulate C3 secretion only in one of the groups studied.

No other statistically significant correlations between TNF-α and the other protein levels were found by us. However, a possibility exists that there is such a significant correlation in the M group, since correlation values are high: positive ones for IgM ($r = 0.815$) and C4 ($r = 0.790$) and a negative one for CIC ($r = –0.809$) (see Table 1, Fig. 4). It is quite possible that this lack of significance is a result of too small a number of patients in this group (4 patients), as described earlier[9]. According to literature data, complement binding to cells can stimulate the production of TNF-α and other proinflammatory cytokines (studies on human kidney tubular cells cultures[6]). Similarly, immunological complexes (both soluble and insoluble) induce rapid secretion of TNF-α by human blood monocytes[7]. Additionally, correlation coefficients calculated for TNF-α and C1i interdependencies were quite high in 3 groups (see Table 1). We found a positive correlation between TNF-α and C1i in the control group ($r = 0.599$) as well as in the DT group ($r = 0.404$) and a negative correlation ($r = –0.410$) in the BC group; however, again, these results were not statistically significant. Studies on more numerous patients would allow verifying these data. For now, it seems reasonable to expect the existence of C1i/TNF-α level interdependencies, since TNF-α induces C1i synthesis in a variety of cell types[16].

Summing up, our studies allowed gathering experimental data on TNF-α as well as on some humoral immunity indeices' levels in a control group and in neoplastic patients. There are sets of data concordant with expectations based on literature reports (negative correlation between TNF-α and IgG in CG; a positive correlation between TNF-α and C3c in the M group). Some results need to be confirmed or excluded by further studies (positive IgM, C4 and CIC correlation with TNF-α in the M group; correlations of TNF-α and C1i--positive in the CG and the DT group and negative in the BC group). However, most of the results are valuable as they allow asking new questions directing our future endeavours.

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