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CRP, TNF α , IL-1ra, IL-6, IL-8 and IL-10 in Blood Serum of Colorectal Cancer Patients

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Blood serum cytokines: TNF α , IL-1ra, IL-6, IL-8, IL-10 as well as CRP were investigated in patients with colorectal cancer, prior treatment and 1, 10 and 42 days after surgery. There was an increase of the levels of CRP, IL-6 and IL-10 in most patients 24 hours after surgery. The levels of IL-1ra were ele-

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vated in patients in stage C and in several patients in stage B of the disease and there was a decrease of circulating TNF α in stage B patients. On day 10 and 42 after surgery, the levels of cytokines followed various patterns. (Pathology Oncology Research Vol 6, No 1, 38–41, 2000)

Introduction

Despite advances in treatment of malignant neoplasms, colorectal cancer remains the malignancy causing difficulties in pre-operative staging. Stages B, C and D, according to modified Dukes classification, are homogeneous from pathological point of view but clinically heterogeneous, what strongly influences prognosis, and results in differences in 5 year survival. The search for new tumour markers continues, with emphasis on various biological parameters such as cytokines, oncogenes, expression of tumour suppressor genes, DNA content,⁴ proliferative index and the tumour doubling time.⁵

Surgical procedures or postoperative inflammatory response trigger release of cytokines and the acute phase response proteins, but the mechanisms of this phenomenon are still under investigation. It is well known that cytokines, such as TNF α ,⁷ IL-1, IL-6,^{2,7} stimulate production of the acute phase response proteins in the liver, and their release observed in post-operative wound healing can be related to rapid local recurrence.^{1,8,9}

The aim of this paper was to describe the levels of circulating pro-inflammatory cytokines: TNF α , IL-6, IL-8, their inhibitors: IL-1ra and IL-10 and acute phase response protein – CRP in colorectal cancer patients before and after surgical treatment.

Material and Methods

Blood serum cytokines (TNF α , IL-1ra, IL-6, IL-8, IL-10) and CRP were assessed in 35 patients with histologically confirmed colorectal cancer, aged 32 to 81 before treatment and 1, 10 and 42 days after surgery. The surgery involved locally radical excision of the colorectal tumour.

Twenty patients in stage B (B1 and B2), 7 in stage C (C1 and C2) and 8 in stage D (D1 according to our own modification of the staging – indicating histologically confirmed infiltration of the adjacent organs and D2 – distant metastases) were under study. None of the patients had shown any symptoms of systemic infection prior to surgery.

Normal ranges of circulating cytokines were set by examining blood sera of 40 healthy volunteers. Blood serum samples were stored at -70°C prior to analysis. The levels of cytokines in sera were assessed using ELISA test by R&D Systems, Inc., Minneapolis, MN, and CRP using ELISA test by Abbott, North Chicago.

The study was approved by the Local Ethics Committee at Maria Sk³odowska-Curie Memorial Cancer Centre in Warsaw.

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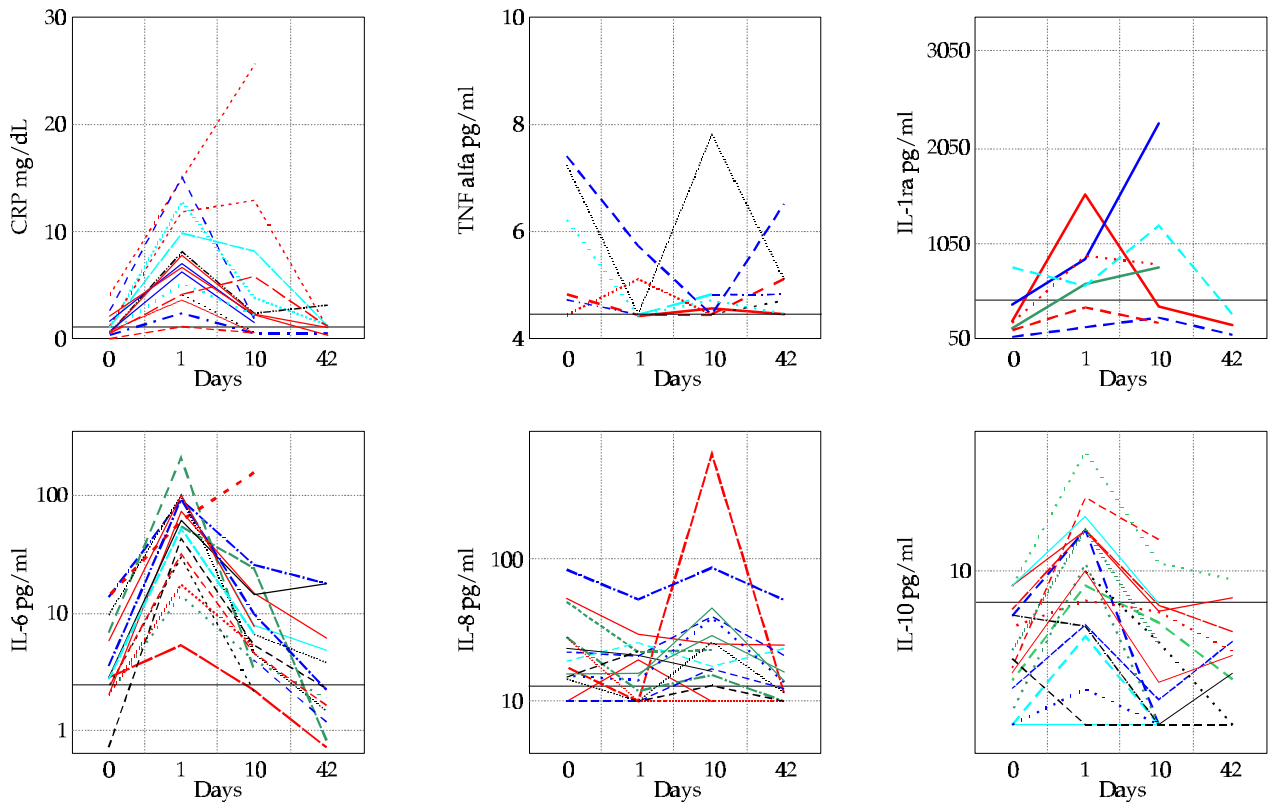


Figure 1. Serum CRP and cytokine levels in patients with colorectal carcinoma in stage B before and after surgery

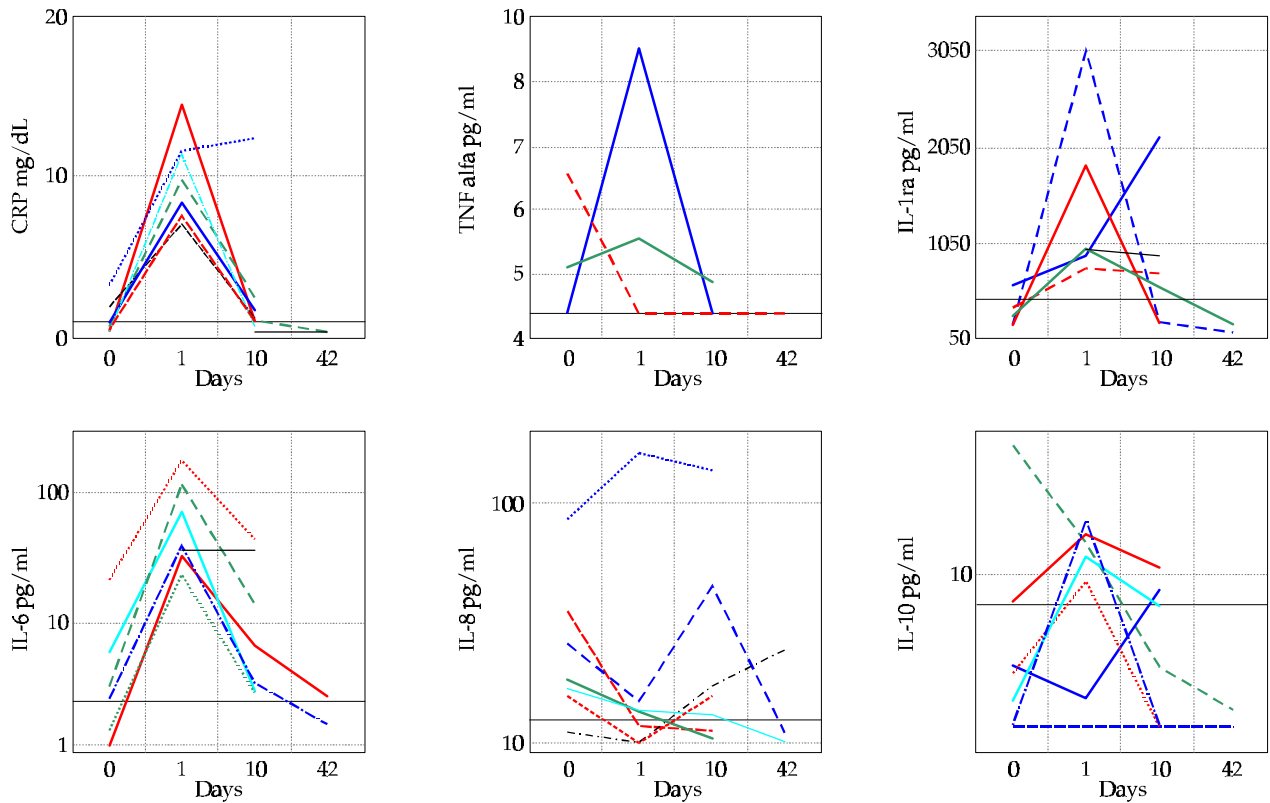


Figure 2. Serum CRP and cytokine levels in patients with colorectal carcinoma in stage C before and after surgery

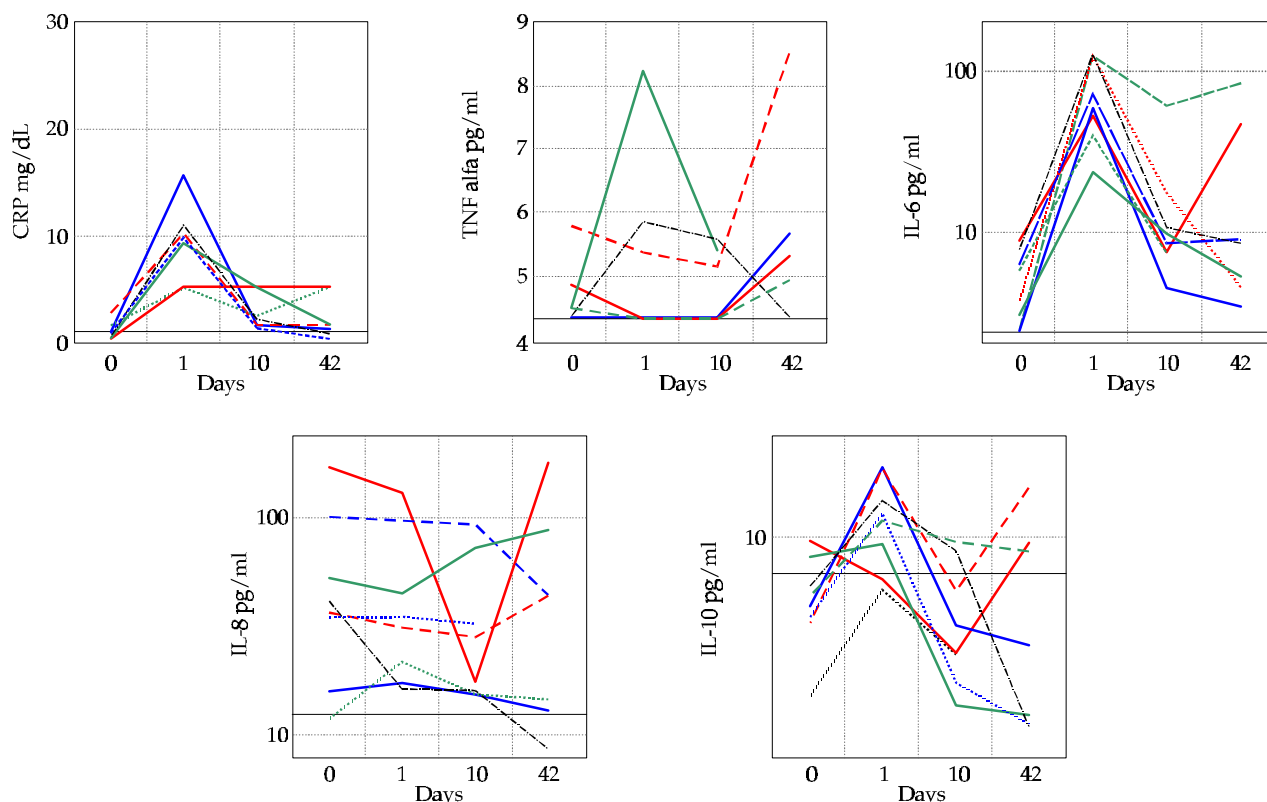


Figure 3. Serum CRP and cytokine levels in patients with colorectal carcinoma in stage D before and after surgery

Results

In the majority of patients in stages B, C and D elevated CRP levels prior to surgery were observed, reaching peak values 24 hours following surgery. The levels of CRP 10 and 42 days after surgery depended on the clinical status of the patient, and were associated with acute and chronic inflammation.

A significant ratio of patients in stage B has shown elevated levels of circulating TNF α , IL-6 and IL-8 already prior to surgery. One day following surgery the highest levels of all cytokines examined were observed for IL-6 (all measurements exceeding normal range) and IL-10 (measurements either exceeding or falling within the normal range). Ten days following surgery, the levels of IL-6 and IL-10 were significantly reduced. In the significant percentage of patients, IL-6 levels were still elevated on 42 day following surgery. However, at this time point only a few patients presented elevated levels of serum IL-10. TNF α and IL-8 levels were usually reduced on the first day following surgery and reached peak values on the 10-th day. On the 42 day, the levels of these two cytokines remained elevated in a significant percentage of patients. The highest levels of IL-1ra were observed on the first and tenth day following surgery (Figure 1).

Seven patients in stage C have shown similar course of serum levels of IL-6, IL-8 and IL-10 to stage B patients. The changes of IL-8 levels were similar in stages B and C. In 6 out of 7 cases in stage C the level of this cytokine, elevated prior to operation, decreased 24 hours after surgery; 10 days after surgery its level increased again, though not always reached the maximum values. The pattern was different just in one case, where IL-8 level increased 1 day after surgery and on the 10th day post-surgery was found to be slightly diminished (down to 137 pg/ml). This patient had non-resectable tumour, metastatic to draining lymph nodes, with no distant metastases at the time of surgery. Liver metastases were detected 3 months later.

In stage C patients' group peak values of IL-1ra were found on the first day following surgery. There were strikingly high levels of IL-8, IL-6 and TNF α in 2 patients. One of these patients developed metastatic liver disease and the other had numerous lymph node metastases (8 lymph nodes involved out of 15 in surgical specimen) (Figure 2).

In 8 investigated patients in stage D, the influence of the surgery on IL-6 and IL-10 levels was similar to that observed in stage B and C. In two patients TNF α levels reached peak values on the first day following surgery, and in the other patients on 42 day. IL-8 levels were high even prior to surgery, and after surgery they followed

nonspecific changes. On 42 day following surgery, in large proportion of patients we have found high levels of the assessed cytokines, especially of IL-8, TNF α and IL-6 (*Figure 3*).

Discussion

The role of cytokines in cancer immunity has been extensively studied in the last few years. In post-operative patients, the reduced percentage and activity of T lymphocytes, NK cells and other symptoms of immunosuppression have been demonstrated. Pro-inflammatory cytokines have been postulated to strongly influence the immunological status of those patients. Among pro-inflammatory cytokines, IL-6 and TNF α play a central role in shock and inflammatory reactions. IL-6 and TNF α stimulate or inhibit the production and activity of cytokines and acute phase proteins, influence receptors' expression. Those multifunctional cytokines also present other activities. IL-6 stimulates T lymphocyte proliferation, influences growth, differentiation and migration of tumour cells, stimulates angiogenesis.

IL-8, another mediator of shock and inflammation also activates neutrophils and stimulates angiogenesis.

IL-10 is an inhibitory cytokine, playing an important role in the regulation of T cell response and inflammatory reactions. IL-10 suppresses the production of pro-inflammatory cytokines (IL-1, IL-6, IL-8, TNF α). An inhibition of IL-10 synthesis may suggest a profound, after shock immunosuppression and predict poor prognosis.^{2,3,6,10}

The results presented here show the patterns of CRP and pro-inflammatory cytokines in the sera of colorectal cancer patients prior and 1, 10 and 42 days after surgical treatment. The majority of patients, regardless of the stage of disease, have shown elevated levels of circulating TNF α , IL-6, IL-8 and CRP prior to surgery. Haupt et al³ have studied a large group of patients subjected to major abdominal surgery and suggest that elevated levels of IL-6 and CRP prior to treatment constitute poor prognostic factors and indicate the possibility of development of post-operative infection. McMillian et al⁶ published similar observations on elevated CRP levels as being poor prognostic factor.

In our studies, there was an increase of the levels of CRP, IL-6 and IL-10 24 hours after surgery in all patients, and of

IL-1ra only in patients in stage C of the disease and a decrease of TNF α in most patients in stage B. Ten and 42 days following surgery, IL-6 serum levels returned to normal values in all patients except for those with stage D disease. The same pattern was observed for IL-1ra in stage B and C patients. The changes of levels of TNF α and IL-10 follow various patterns. The level of IL-8 depended on the stage of disease, and the most characteristic pattern was observed in stage B patients' group (the largest group of patients) – where we observed a decrease after first 24 hours and an increase 10 days following surgery. Similar patterns of cytokine levels were reported by other authors.^{2,7,9}

The preliminary results presented here comprise the initial stage of a study on the influence of surgery on the levels of circulating CRP and pro-inflammatory cytokines and on their prognostic value.

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