# Acute phase proteins and recombinant IL-2 therapy: prediction of response and survival in patients with colorectal cancer

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## SUMMARY

Twenty-four patients with metastatic colorectal cancer were treated with recombinant IL-2 (rIL-2) by continuous intravenous infusion for 5 days ($18 \times 10^6$ $U/m^2$ per 24 h), followed by three injections of 5-fluorouracil (600 $mg/m^2$) and folinic acid (25 $mg/m^2$) at weekly intervals. The response to treatment was assessed using standard UICC criteria (partial or complete response, stasis or progression of disease). The serum concentrations of the acute phase proteins; C-reactive protein (CRP), retinol binding protein (RBP), $\alpha_1$-antitrypsin ($\alpha_1$-AT), transferrin (TF) and albumin were measured. A response to therapy occurred in the tumours of seven (29%) of the 24 patients (two complete and five partial responses). All patients who demonstrated a response to treatment had a serum albumin level of $> 37$ g/$l$ and a CRP level of $\leqslant 10$ mg/$l$. In contrast, of the 17 patients who did not respond to therapy, 12 (71%) had a serum albumin of less than 37 g/dl and a CRP of greater than 10 mg/$l$. Examination of the survival times of the 12 patients who had a pretreatment serum albumin level of less than 37 g/$l$ revealed that all had died within 12 months of cessation of therapy. However, 58% of patients with pretreatment serum albumin levels of greater than 37 g/$l$ survived for longer than 12 months. These results have shown that (i) patients who respond to rIL-2-based therapy and (ii) those patients who have prolonged survival times, can be identified by pretreatment measurement of serum levels of acute phase proteins.

**Keywords** IL-2 therapy   acute phase response   response and survival

## INTRODUCTION

Colorectal cancer is a major cause of morbidity and mortality in the UK. The corrected 5 year survival is approximately 40–50% [1] and has remained static during the last 40 years [2]. The majority of the patients who die do so from metastatic disease, with the liver being most commonly affected. Surgical resection of metastatic disease, particularly hepatic metastases, is being undertaken in some centres, but only small numbers of patients (less than 15%), will benefit with a prolongation of survival [3]. Chemotherapy, therefore, has been used to treat patients with metastatic colorectal cancer. Currently, the combination of 5-fluorouracil (5-FU) and leucovorin (LV) appears to be most effective in reducing tumour bulk and prolonging survival [4]. However, the response rates to chemotherapy are still disappointing and there is, therefore, an urgent need to develop new modalities of therapy. Recent interest, therefore, has focused on the use of

biological response modifiers, in particular, recombinant IL-2 (rIL-2).

IL-2 is a 15·5-kD protein which is derived from T helper cells which has well described effects on the immune system. In particular, it stimulates a variety of host anti-tumour defence mechanisms such as natural cytotoxicity, and causes the release of several different cytokines which themselves may have important anti-tumour effects [5–7]. Although the administration of rIL-2 to animals can result in the eradication of a range of metastatic tumours [8,9], the results in the treatment of human cancers have been less impressive. The solid tumours most responsive are metastatic melanomas and renal cell cancers, with response rates of up to 60% being reported when rIL-2 is used in combination with chemotherapeutic agents [10]. Similarly, pilot studies in patients with metastatic colorectal cancer had previously documented a response rate of approximately 30% when rIL-2 was used in combination with 5-FU [11]. However, rIL-2 therapy may be associated with a substantial morbidity [12], and attempts have been made, therefore, to predict which patients would respond to rIL-2-based therapy using a range of clinical, laboratory and

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immunological indices. However, none of these has proven to be a reliable indicator of response or survival [13].

Interest has focused on the function of the acute phase proteins in patients with malignant disease. Previous studies have shown that elevated serum levels of C-reactive protein (CRP) are associated with a poor prognosis [14,15], and reduced serum albumin levels are also associated with a worse prognosis [16,17]. We have documented previously, in a pilot study, that the pretreatment serum level of the acute phase protein, CRP, was lower in patients who responded to rIL-2 therapy than in patients who did not respond [18], and similar findings have been reported in patients with metastatic renal cell cancer [19].

The aim of the present study, therefore, was to determine if the pre-treatment serum levels of a range of acute phase proteins (albumin, $\alpha_1$-antitrypsin ($\alpha_1$-AT), retinol binding protein (RBP), transferrin (TF) and CRP) could, (i) predict which patients with metastatic colorectal cancer would respond to immunochemotherapy (rIL-2/5-FU/LV), and (ii) if the serum levels of these acute phase proteins would identify those patients most likely to benefit from such therapy.

## PATIENTS AND METHODS

### Patients
Twenty-four patients with metastatic or locally advanced colorectal cancer (Dukes C or D) were entered into the study. All patients had an ambulatory performance status (Eastern Co-operative Oncology Group 0-1, Karnofsky >80%) and had a life expectancy of greater than 3 months. Renal and liver function tests were within normal limits (unless due to the malignancy) and no patient had received chemotherapy, radiotherapy or immunotherapy in the 4 weeks before entering into this study. The study was approved by the Joint Ethical Committee of the Grampian Health Board and the University of Aberdeen. All patients gave signed informed consent before participation in the study in terms of increased survival.

### rIL-2/chemotherapy schedule
A constant intravenous infusion of rIL-2 (Proleukin, Eurocetus, Amsterdam, The Netherlands) was given at a dose of $18 \times 10^6$ U/m$^2$ per 24 h for a total of 120 h. Forty-eight hours after cessation of rIL-2 infusion patients received a bolus i.v. injection of 5-FU (600 mg/m$^2$) and LV (25 mg/m$^2$). The chemotherapy was repeated at weekly intervals for 3 weeks. At the end of this 4-week period (comprising one cycle of therapy) the patients' disease was re-assessed. If there was evidence of either stasis or a response (complete or partial), patients received further cycles of therapy up to a maximum of six. However, if there was progression of disease patients were withdrawn from the study.

### Assessment of tumour response
The disease was measured either clinically (if evaluable) or by standard radiological imaging techniques—ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI). Tumour responses were assessed according to standard WHO criteria [20]. A complete response (CR) was defined as the disappearance of all known lesions, determined by two observations not less than 4 weeks apart. A partial response (PR) was defined as a greater than 50% reduction in tumour

size (multiplication of the longest diameter by the longest perpendicular diameter); with multiple lesions, a PR was a greater than 50% decrease in the sum of the products of the perpendicular diameters of the multiple lesions, determined by two observations not less than 4 weeks apart. Stable disease (SD) was defined as less than a 50% decrease in total tumour size or less than a 25% increase in the size of the measurable lesions. Progressive disease (PD) was more than a 25% increase in the size of any measurable lesion or the appearance of new lesions.

### Timing of blood samples
Peripheral blood was collected without the use of a tourniquet before commencement of rIL-2/chemotherapy. Blood was allowed to clot and then spun at 1000 g for 10 min. Serum was separated and stored at $-20^\circ$C until analysis.

### Measurement of acute phase proteins
Concentrations of serum albumin were measured by dye binding (bromocresol green) on a Technicon DAX-72 discrete autoanalyzer (Bayer Instruments, Basingstoke, UK) using Bayer reagents. Concentrations of CRP and $\alpha_1$-AT were measured by rate nephelometry [21] on a Beckman ICS Analyzer II system (Beckman Instruments (UK) Ltd, High Wycombe, UK) using Beckman ICS reagents. The same instrument was employed, using Behring antibody and standard reagents (Behringwerke AG, Marburg, Germany), for measurement of RBP. Transferrin was measured by turbidimetry on a Technicon RA-1000 discrete autoanalyzer (Bayer), using Technicon reagents.

### Statistical analysis
Statistical comparisons of pretreatment serum levels of the acute phase proteins were made between patients who responded to the rIL-2-based therapy (complete and partial responses) and those patients who did not respond (stasis and progression of disease), using the Mann–Whitney $U$-test. Kaplan–Meier survival plots were evaluated using the method described by Dinse et al. [22] for the detection of time ranges over which survival curves significantly differed. $P < 0.05$ was accepted as significant.

## RESULTS

### Responses to rIL-2/5-FU/LV
A response rate of 29% (7/24) was observed, with five patients having a partial and two a complete response. In seven patients there was stasis of disease and a further 10 had progression of disease. For analysis of the data, patients were grouped as either 'responding patients' (complete or partial response), and 'non-responding patients' (stasis or progression of disease).

### Pretreatment acute phase proteins and response to therapy
*C-reactive protein.* The pretreatment serum levels of CRP in the seven responding patients were all 10 mg/l or less, ranging from 1 to 10 mg/l, with a median of 3 mg/l. However, in the 17 non-responding patients, the pretreatment levels of CRP varied widely (range 3–116 mg/l; median 50 mg/l), $U = 8.5$, $P = 0.003$ (Fig. 1).

*$\alpha_1$-antitrypsin.* The pretreatment serum concentration of $\alpha_1$-AT was significantly lower in the responding group
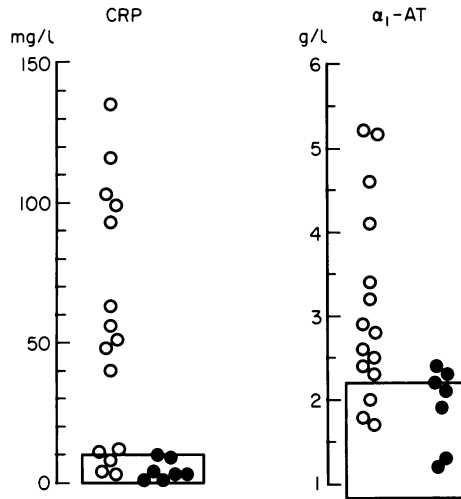
Fig. 1. Pretreatment levels of C-reactive protein (CRP) and $\alpha_1$-antitrypsin ($\alpha_1$-AT) in the responding patients were significantly reduced compared with non-responding patients ($P = 0.003$ and $P = 0.01$, respectively). Boxes represent normal ranges. O, Non-responding patients; ●, responding patients.

of patients (range 1·2–2·4 g/l; median 2·1 g/l), compared with the non-responding patients (range 1·7–5·2 g/l; median 2·8 g/l), $U = 16$, $P = 0.01$ (Fig. 1).

*Albumin.* The pretreatment serum albumin levels in the responding patients ranged from 38 to 44 g/l (median 40 g/l). In contrast, the serum albumin in the non-responding group of patients ranged from 26 to 41 g/l (median 34 g/l), $U = 16$, $P = 0.01$ (Fig. 2).

*Transferrin.* The pretreatment levels of transferrin were also lower in the responding patients (range 1·7–3·3 g/l; median 2·8 g/l), compared with the non-responding group (range
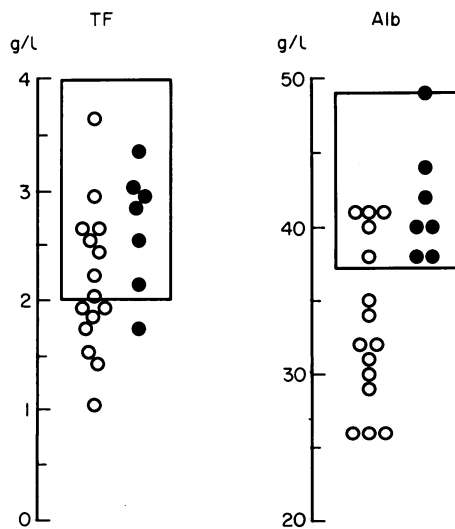


Fig. 2. Pretreatment levels of albumin (Alb) were significantly higher in the responding than the non-responding patients ($P = 0.01$). There were no significant differences in transferrin (TF) concentrations between either group of patients ($P = 0.09$). Boxes represent normal ranges. O, Non-responding patients; ●, responding patients.
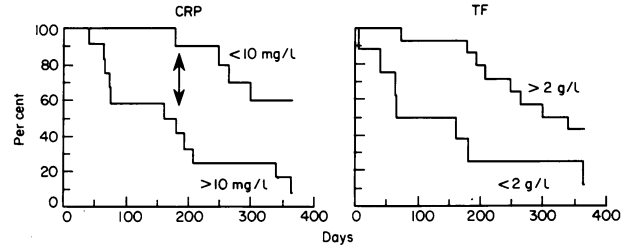


Fig. 3. Kaplan–Meier survival curves for groups of patients grouped according to their pretreatment serum concentrations of C-reactive protein (CRP) and transferrin (TF). The arrows indicate the time after which the curves for CRP diverged (95% upper confidence bound). There were no significant differences in the transferrin (TF) survival curves.

1·0–3·6 g/l; median 2·1 g/l), but this did not achieve statistical significance ($U = 26$, $P = 0.09$) (Fig. 2).

*Pretreatment acute phase protein concentrations and survival*

*C-reactive protein.* The survival of patients with pretreatment serum CRP concentrations of 10 mg/l or less was compared with those patients with levels greater than 10 mg/l (10 mg/l is the upper limit of normal for serum CRP levels in our laboratory). The survival curves for the two groups of patients are shown in Fig. 3. Analysis of these survival curves demonstrated a significant prolongation of survival in patients with the lower pretreatment CRP level, the curves diverging after 161 days (95% upper confidence bound).

*Transferrin levels.* The effect on survival of pretreatment transferrin concentrations of either less than 2 g/l or greater than 2 g/l is shown in Fig. 3. Although there was a trend to a prolonged survival in patients with the higher transferrin concentrations, the survival curves were not significantly different.

*Albumin.* The survival of patients with a pretreatment serum albumin concentration of less than 37 g/l was compared with those with concentrations of greater than 37 g/l (the lower limit of serum albumin concentration being 37 g/l in our laboratory). The survival curves for these patients (Fig. 4) demonstrate a significant prolongation of survival in those patients with pretreatment albumin concentrations of greater than 37 g/l, the curves diverging after 161 days (95% upper confidence bound).
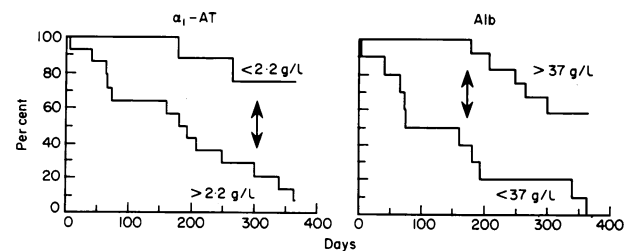


Fig. 4. Kaplan–Meier survival curves for groups of patients grouped according to their pretreatment serum concentrations of $\alpha_1$-antitrypsin ($\alpha_1$-AT) and albumin (Alb). The arrows indicate the time after which the curves for C-reactive protein (CRP) diverged (95% upper confidence bound).

$\alpha_1$-*antitrypsin*. The survival of patients with pretreatment $\alpha_1$-AT concentration of less than $2.2\,g/l$ was compared with patients with levels greater than $2.2\,g/l$ ($2.2\,g/l$ being the upper limit of normal). The survival curves (Fig. 4) show a longer survival in those patients with a pretreatment $\alpha_1$-AT of less than $2.2\,g/l$, compared with patients with lower concentrations, the curves diverging after 300 days (95% upper confidence bound).

## DISCUSSION

The acute phase response occurs in response to damage of body tissues as a result of inflammation, trauma and malignant disease. It is characterized by the alteration in production and secretion of more than 30 different plasma proteins. These proteins are termed the acute phase proteins [23], and examples of the more commonly studied ones are CRP, $\alpha_1$-AT, TF and albumin. In man, during the acute phase response, the plasma concentrations of these proteins change, predominantly due to alterations in hepatic synthesis and/or secretion [24]. For example, the plasma concentrations of CRP and $\alpha_1$-AT increase, CRP concentrations by up to a 1000-fold, but the plasma concentrations of transferrin and albumin fall during the acute phase response [25]. However, the fall in circulating albumin levels may also be explained, in part, by changes in albumin catabolism and/or increased losses into the extravascular compartment due to an increased microvascular permeability [24,26]. The functions of these different proteins are variable, wide-ranging and, as yet, inadequately determined. However, modulation of the immune response (e.g. enhancement of natural cytotoxicity), mediation of the inflammatory response, free radical scavaging and enzyme inhibition, as well as opsonization of invading organisms prior to phagocytosis, have all been described [27,28]. The biological control of the acute phase response is highly complex. Previous studies have shown that IL-1, IL-6, tumour necrosis factor-alpha (TNF-$\alpha$) and corticosteroids all play an important role in the metabolism of acute phase proteins [29,30]. Furthermore, it has been shown that the various acute phase proteins differ in their metabolic responses to specific cytokines, suggesting that there may be a precise regulation of each acute phase protein by the interaction of specific cytokines [31].

The results from the study reported here have confirmed and extended the preliminary observations we made previously regarding serum levels of CRP and the response to rIL-2-based treatment [18]. To the best of our knowledge it is the most detailed study in man, to date, of the metabolism of acute phase proteins in a defined tumour type, and its relationship to response to specific therapy and subsequent survival. Our study has demonstrated that patients who responded (complete and partial responses) to rIL-2-based therapy had significantly reduced pretreatment CRP and $\alpha_1$-AT serum concentrations compared with non-responding patients (stasis or progression of disease). In addition, the serum levels of albumin and transferrin were higher in the responding than in the non-responding patients. Such a pattern of plasma levels of acute phase proteins, therefore, suggests that patients with metastatic colorectal cancer who have an active on-going acute phase response before therapy with rIL-2, will not respond to such treatment. In contrast, patients who do not have an on-going acute phase response before starting

treatment demonstrate a response to rIL-2 therapy (in terms of reduction in tumour volume). Other workers have also shown that in patients with metastatic renal cancer, the pretreatment plasma levels of CRP were significantly lower in responding compared with non-responding patients [19]. However, they did not measure other acute phase proteins. It is well recognized, on the other hand, that the response of a tumour, in terms of a reduction in tumour volume, does not necessarily mean that the patient's survival will be prolonged.

The serum concentrations of certain acute phase proteins, primarily CRP, have been shown previously to be prognostic indicators in patients with metastatic malignancies, e.g. colorectal, breast and stomach cancers [32]. The results from our study demonstrate that placing patients into two well defined groups (with pretreatment plasma levels below or above normal limits), the serum levels of the acute phase proteins were reliable prognostic indicators and predictors of likely response to combination therapy with biological response modifiers. The presence of a lower pretreatment serum level of CRP and $\alpha_1$-AT or a higher pretreatment level of albumin or transferrin, was associated with a prolonged survival.

The reasons for these differences remain unclear, but we have previously suggested that high levels of CRP may be an indicator of the tumour burden or the biological aggressiveness of the tumour [18]. The role of CRP also requires careful consideration. Although some studies have shown that CRP can enhance host defence mechanisms, e.g. lymphocyte proliferation and natural cytotoxicity [33,34], other studies have suggested that CRP may interfere with the immunological mechanisms involved in the anti-tumour activity of rIL-2 [35], thus possibly explaining why patients with high pretreatment CRP levels do not respond to such therapy. Recent interest has also focused on the relationship of IL-6 (a key modulator of hepatocyte production of CRP) to the acute phase response in patients with cancer. We have previously shown that patients who responded to rIL-2-based therapy had lower pretreatment levels of IL-6 compared with non-responding patients [36], and similar findings have been reported in patients with metastatic renal cancer [19]. In addition, IL-6, although stimulating host anti-tumour defence mechanisms, has also been shown to be an autocrine growth factor for cancer cells, most notably renal cancer cells *in vitro* [37] as well as *in vivo* [38]. Thus, patients with malignant disease who have an established autologous inflammatory/immune response, which presumably is ineffective, fail to benefit from an enhancement of this ongoing response with rIL-2, which may either inhibit further tumour-specific or directed anti-cancer responses and/or possibly stimulate tumour cell growth through *in situ* release of tumour-promoting cytokines. In contrast, in those patients who are not demonstrating an inflammatory/immune response, in spite of a significant and comparable tumour burden, stimulation of their immune systems with rIL-2 has a beneficial role in terms of well demonstrated anti-tumour effects—measurable reduction of tumour volume and clinically demonstrable prolongation of survival. Presumably in this latter group, selective enhancement of anti-tumour responses overcomes any possible tumour-promoting effects.

In conclusion, we have demonstrated that the measurement of the serum levels of acute phase proteins in patients with

metastatic malignancy enables a prediction to be made as to which patients would benefit from rIL-2-based therapy, in terms of reduction in tumour volume and likelihood of prolongation of survival. Such measurements, which are cheap and readily available in most hospital laboratories, enable a clinician to target such therapy to those most likely to benefit, thus reducing therapeutic costs and preventing unnecessary morbidity. Further studies are now required to evaluate further the complex interactions of cytokines, acute phase proteins and the immune response in patients with cancer.

## ACKNOWLEDGMENTS

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