Steep elevation of blood interleukin-6 (IL-6) associated only with late stages of cachexia in cancer patients

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ABSTRACT. Changes in blood cytokines of 28, consenting, terminally ill cancer patients were studied to determine a relationship between cachexia and changes in cytokine levels. Levels of PTHrP and five types of cytokines considered to be associated with cachexia, TNFα, IL-1β, IL-6, IFNγ and LIF, were measured during routine blood examination and were compared with clinical findings. With the exception of TNF-α, which was detected in one patient, only IL-6 was detected in all 28 patients recruited in this study. Ten patients showed a sharp elevation of IL-6 just before death, following a 40-day period in which IL-6 was continually detected in the blood. In six out of these ten patients, levels of 100pg/mL or more of IL-6 were detected in the week prior to death. The average period between detection of these levels of IL-6 and death was 2.0 days. Progression of carcinoma is believed to induce a variety of cytokines, which cause loss of appetite, weight loss, tissue wasting, and finally patients may become cachectic. Of the six cytokines studied during this test, only the level of IL-6 was significantly elevated, and this sharp rise occurred approximately one week before patients died. We conclude that IL-6 increases only gradually during the early stages of cachexia but then shows a sudden and steep rise just before death.

Keywords: Cytokines, IL-6, cachexia, cancer

INTRODUCTION

Cancer cachexia is a syndrome characterised by marked weight loss, anorexia, asthenia and anaemia. The cachectic state is invariably associated with the presence and growth of the tumour and leads to malnutrition status due to the induction of anorexia or decreased food intake [1]. An accelerated starvation state is brought about in the patient because of the competition for nutrients between the tumour and the body. Cachexia is an important factor associated with the prognosis and Quality of Life (QOL) of cancer patients, and, as a definition of cachexia has not been established using a medically objective marker, establishing the prognostic factors of cancer cachexia may help to improve the QOL of terminally ill cancer patients.

Many researchers are currently focusing their attention on the relationship between cachexia and cytokines. Cytokines are intercellular signal transmission factor proteins, and have been implicated in cachexia [1]. The physiological activities of cytokines were first described in the early '80s, their genes were then cloned, and their role as a control factor of the immunohaematology system are now widely recognised. Since the late '80s, results from animal experiments have suggested that some cytokines affect cachexia. For example, Cerami and Beutler showed in 1985 that cachectin (murine tumour necrosis factor) possibly plays a central role in evoking a state of cachexia in mice [2]. In 1987, one report showed that cachectic conditions were induced by implanting Chinese Hamster Ovary (CHO) cells, derived from a TNF-α gene into an immunodeficient nude mouse [3]. In 1991, another report [4] showed that nude mice, implanted with the human melanoma cell line, SEKI, expressed large amounts of LIF (leukaemia inhibitory factor), resulting in cachexia. In 1991, another article reported that weight loss was correlated with IFN-γ in Lewis lung tumours of mice, and anti-IFN antibody inhibited a decrease in body fat [5]. In 1995, it was reported that a model animal with a colon 26 adenocarcinoma showed an increase in blood Interleukin-6 levels (IL-6) and progressive cachexia [6]. In the same year (1995), treatment of rats with genetic recombinant Interleukin-1 (IL-1β) was reported to cause inhibition of eating behaviour. These findings suggest that there is a relationship between cytokines and cachexia, and have led to recent clinical studies which investigate this relationship.

As stated above, various cytokines are suspected of being involved in cachexia. In this retrospective study, we measured and analysed changes in some of these cytokines in the plasma of terminally ill cancer patients. In the latter part of the investigation, efforts were focused on IL-6 in particular.

PATIENTS AND METHODS

Twenty-eight cancer patients, with several types of carcinoma; nine breast cancers, four oesophageal cancers (male), four lung cancers (male), one CCC (cholangiocel-

Table 1

No.	Sex	Age	Type of Carcinoma	Primary/local recurrence	Distant metastasis	Treatment goal	Max IL-6*	Days till death*
1	M	81	Oesophageal	+	–	palliative	99.4	13 days
2	M	51	Pancreatic	+	–	palliative	14.9	NA
3	M	76	Oesophageal	+	–	palliative	13.5	5 days
4	F	76	Breast	–	+	palliative	870	1 day
5	F	43	Cervical	–	–	curative	53.9	NA
6	F	51	Breast	–	+	palliative	145	3 days
7	M	57	Gastric	+	–	palliative	21.1	NA
8	M	65	Oesophageal	+	–	palliative	292	1 day
9	F	58	Breast	–	+	palliative	4.6	6 days
10	M	67	Lung	+	–	curative	138	NA
11	M	82	Lung	+	–	palliative	25.6	NA
12	F	85	Pancreatic	+	–	palliative	38.2	NA
13	F	66	Breast	–	+	palliative	7.8	3 days
14	M	90	Oesophageal	+	–	palliative	124	2 days
15	F	60	Breast	–	+	palliative	48.9	92 days
16	F	52	Cervical	+	+	palliative	686	1 days
17	F	68	Breast	–	+	palliative	83.3	29 days
18	M	46	HCC	+	–	palliative	60.3	NA
19	F	38	Breast	–	+	palliative	228	1 days
20	M	75	CCC	+	+	palliative	41.0	18 days
21	F	41	Breast	–	–	curative	6.8	NA
22	F	53	Breast	–	+	palliative	752	7 days
23	M	74	Gastric	+	–	palliative	49.9	5 days
24	M	54	Brain Tumour	+	–	palliative	88.3	74 days
25	M	75	HCC	+	–	palliative	82.0	2 days
26	M	76	Lung	+	+	palliative	678	1 days
27	M	78	Gastric	+	–	palliative	15.7	64 days
28	M	54	Lung	+	–	palliative	117	2 days

*IL-6: pg/mL. *Days until death: number of days between date of max IL-6 and death. *NA: either survived the trial or was transferred to another institution.

lular carcinoma) (male), three gastric cancers (male), two HCC (hepatocellular carcinoma) (male), two pancreatic cancers (one male, one female), one brain tumour (male) and two cervical cancers, gave their consent to participate in this study, which took place between January 2002 and October 2003. The average age was 65.2 years (for details see *table 1*).

Out of those 28 patients, three patients were admitted to hospital for primary treatment; five patients with local advanced cancer had radical treatment, seven patients with metastases had treatment for local control, and 13 patients with metastases were treated only to control symptoms. Treatment goals for each individual are shown in *table 1*.

All cytokines and parathyroid-related peptide (PTHrP) were measured using the same methods. There follows a description of these methods using IL-6 as an example.

Two ml blood samples were taken during routine blood checks. These samples were stored at -70 °C; after centrifugation. IL-6 was measured in compliance with CLEIA (chemiluminescent enzyme immunoassay) methods using the Lumipulse® *f* automatic analyser. Ferrite particles (0.3 μm in diameter) were coated with anti-IL-6 monoclonal antibody. The blood samples (50 μL) were added to the ferrite suspension and incubated at 37 °C; for 10 minutes. The suspension was washed three times with buffer by magnetic separation. ALP-labeled anti-IL-6 monoclonal antibody (250 μL) was then added and the suspension was again incubated for 10 minutes at 37 °C; and washed with buffer. Lumipulse® substrate solution (AMPPD chemiluminescent) was then added to the reaction and the mixture was stirred at 37 °C, for five minutes.

Physical observation, PS, CRP and other haematology data were entered into a database along with the cytokine data. PTHrP and five types of cytokines, namely TNF-α, IL-1β, IL-6, IFN-γ and LIF, were measured in the first eight cases. After the 8th case, only IL-6 was measured.

The collection of blood samples from terminally ill cancer patients, whether consensual or not, may be considered to be unethical, especially if the patient is in the final stages of their illness. It is difficult to enforce a regular blood-sampling regime in these circumstances, and for this reason there was no protocol for the collection of blood samples in this study. Instead, blood was collected coincidentally at times when the attending physician saw it necessary to take a blood sample for some other reason. Consequently, any blood samples taken immediately prior to death are purely by chance.

RESULTS

When the results for the first eight cases were collected, almost no TNF-α, IL-1β, IFN-γ, LIF, or PTHrP was detected in the blood. IL-6 was detected in all cases, at a concentration of more than 10pg/mL *(table 2)*. TNF-α, was detected in one case (14pg/mL), see *table 2*.

Table 1 shows data for all 28 patients including sex, age, type of carcinoma, primary/local recurrence, distant metastasis, treatment goal, maximum level of IL-6, and the number of days until death from the date of maximum IL-6 levels.

Table 2
Serum concentrations of cytokines and PTHrP

Patient No	IL-6 (pg/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	IFN-γ (IU/mL)	PTHrP (pmol/L)
1	99.4	<10	14	<0.1	8.1
2	14.9	<10	<5	0.1	17.6
3	13.5	<10	<5	<0.1	0.7
4	59.3	<10	<5	<0.1	0.2
5	53.9	<10	<5	<0.1	–
6	31.2	<10	<5	0.2	–
7	21.1	<10	<5	<0.1	–
8	292	<10	<5	<0.1	–

A quantitative analysis of IL-6 was performed twice or more in 16 cases and five times or more in six cases.
Ten patients, who had IL-6 in their blood continuously for 40 days prior to death, showed a rapid increase in IL-6 just before death *(figure 1)*. Six of these ten patients showed an IL-6 concentration of 100 pg/mL or more in their blood eight days before death. The average period from detection of 100 pg/mL or higher until death was 2.0 days, with a standard deviation of 1.5 days.
Several patients recorded a maximum IL-6 level several days to months prior to death. These patients were discharged from hospital and so the concentration of IL-6 in their blood before death is not known as they passed away off-site.
None of the patients who survived the trial and who were discharged or transferred, showed a high IL-6 level of 100 pg/mL or more.
Table 3 shows data for 10 cases including cause of death, maximum IL-6, CRP and days until death.

DISCUSSION

It has been assumed that cytokines, including TNF-α, IL-1β, IL-6, IFN-γ, and LIF could cause cachectic symptoms such as loss of appetite, and the massive loss of both adipose tissue and skeletal muscle mass [7]. However, our study did not show any significant increase in blood cytokines apart from IL-6. Among references pertaining to the measurement of cytokines in cancer patients, many studies have reported that TNF-α, a cytokine other than IL-6, increased significantly in cancer patients compared with healthy subjects, and some reports showed a significant increase in IL-1β or IL-8. Therefore, cytokines other than IL-6 may possibly increase in end-stage cancer patients, however this was not shown by the present study.

In the current study, the concentration of IL-6 was seen to rise gradually during the early stages of cachexia followed by a sharp increase in the week prior to death. Maltoni *et al.* measured blood IL-6 in 61 patients with possible cachexia induced by various carcinomas, and analysed the relationship between weight loss and appetite loss [8]. Although biased due to a small amount of evidence Maltoni *et al.* found that the average IL-6 was 0.2 ± 1.4 (pg/mL) and concluded that there was no relationship between these two factors. In other words, they agree with the results of this study in saying that there is no association between cachexia and an increase in IL-6, at least not in early cachexia.

The study of blood IL-6 in gastric cancer patients by Wu *et al.* [9] reported that the more advanced a patient's stage, the higher the probability of IL-6 rising above 10 pg /mL. Blood IL-6 was measured in 14 patients at an interval of

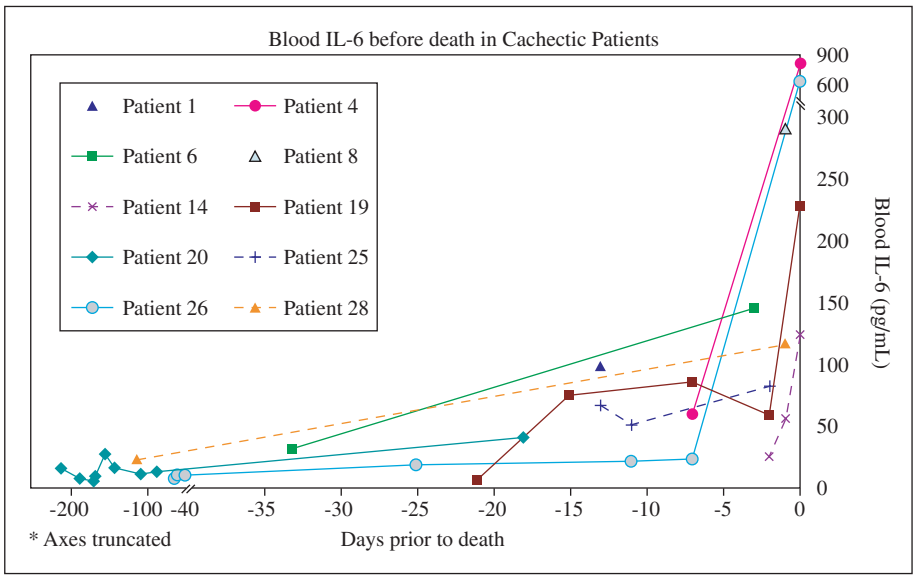

Figure 1

Table 3

Patient No.	Cause of death	Max IL-6 (pg/mL)	CRP (mg/dL)	Days until death
1	Cancer related	99.4	8.4	13
4	Cancer related	870	4.6 (*taken 4 days prior)	0
6	DIC	145	19.6	3
8	CL	292	9.8	1
14	Cancer related	124	1.7	0
19	Pneumonia	228	30.2	0
20	Cancer related	41	7.2 (*taken 21 days prior)	18
25	Cancer related	82	No result	2
26	Cancer related	678	9.5	0
28	Cancer related	117	12.4	1

DIC=Disseminated intravascular coagulation CL=Carcinomatous lymphagiosis • CRP value measured same day as max IL-6, unless otherwise stated.

between 10 and 52 months. All 10 disease-free survivors showed a decrease in blood IL-6, whereas 4 patients relapsed, showing an increase in blood IL-6. The researchers concluded that the blood IL-6 level is a possible prognostic factor.

Martin *et al.* measured blood IL-6 in 58 patients diagnosed with lung cancer, and observed the progress of two groups: 42 patients with less than 130 pg /mL in one group, and 16 patients with 130 pg /mL or more in the other. They reported that the patients with 130 pg /mL or more of IL-6 in their blood had a shorter median survival period [10].The hypothesis for the current study was that there would be a gradual increase in blood IL-6 in end-stage cancer patients as they neared death. However, the level of blood IL-6 was shown to only increase rapidly just before death. IL-6 must be a prognostic factor, but its clinical significance may be very low in the earlier stages of cancer. Nakano *et al.* [11] measured IL-6 in the blood of 25 patients with malignant pleural mesothelioma. No difference in survival rate was reported between patients either side of a 100 pg/mL cut-off point. The findings of Nakano *et al.* cannot be directly compared with Martin's study, as the latter used cut-off values of 130 pg/mL. This report also suggests that since changes in blood IL-6 are affected by the type of carcinoma, mode of progression and the presence of inflammation, life expectancy cannot be predicted simply by using a cut-off value for blood IL-6.

Mantovani *et al.* [12] measured IL-6 in blood before and after treatment in 16 lung cancer patients who were unsuitable for surgery. They reported that the average IL-6 level before the chemotherapy was 110 ± 33.18 (pg/mL). From the findings of the current study and others, blood IL-6 values of 130 pg/mL or higher may indicate extremely advanced terminal stages of cancer.

IL-6 is a cytokine that has been studied as a growth-promoting factor, and may have a main role in inflammation as well as having various other actions. It is highly possible that infection is responsible for an increase in IL-6 in the blood in some end-stage cancer patients, who are susceptible as a result of lowered immunity. In a study by Kobayashi [13] in which IL-6 was measured before and after treatment with antibiotics in 19 gynaecological cancer patients with infections, IL-6 levels in the group that had been treated with antibiotics were compared with those of the group that had not been treated. IL-6 level stayed at 100 pg/mL or less in all cases with or without treatment. This indicates that IL-6 levels are not affected by infection. A sudden and steep increase in IL-6 just before death, as was seen in the current study, was not observed by Kobayashi.

In a study in which blood IL-6 was measured in six patients suffering from severe acute pancreatitis, a condition with high mortality, Ogura *et al.* [14] indicated that blood IL-6 was found to be 200 pg/mL or more in all samples taken at the time of admission to hospital or on entering ICU. Invasive surgery was also said to cause an increase in blood IL-6. Ogura's findings suggest that severe infection and possibly surgery-induced inflammation may cause IL-6 levels to rise in the blood. During the present study, one patient (Patient 19) died from a severe infection (pneumonia). This patient had a high CRP (30.2 mg/dL) and an extremely high blood IL-6 concentration (228 pg/mL). In contrast to this, high levels of CRP did not necessarily coincide with a high concentration of IL-6 in other patients *(table 3)*, suggesting that only severe infections bring about steep increases in IL-6 levels as well as raising CRP. In this study, a high level of IL-6 in the blood (200 ng/mL or more) was seen to indicate a high likelihood of death within a few days following measurement. Therefore, high IL-6 values can be considered to be a prognostic factor for death in terminally ill cancer patients. Such information is very important, especially for the families of patients and there is the possibility that such measurements may provide an early warning and allow families to prepare.

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