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Tumour Necrosis Factor-α, Interleukin-1β and Interleukin-6 in Patients With Renal Cell Carcinoma

Christine Dosquet, Antoine Schaetz, Claire Faucher, Eric Lepage, Jean-Luc Wautier, François Richard and Jean Cabane

Patients with renal cell carcinoma (RCC) can exhibit fever, weight loss and increases in acute phase proteins. Interleukin (IL)-1, tumour necrosis factor (TNF) and IL-6 are considered major mediators of local and systemic inflammation. We measured plasma IL-1β, TNF-α (immunoradiometric assay) and IL-6 (ELISA) in 78 consecutive patients with untreated RCC and in 56 normal subjects. IL-6 plasma levels were higher in patients with RCC (mean 24.2 pg/ml, 11.1–37.3, 95% confidence interval) than in normal subjects (11.6 pg/ml, 10.1–13.1, $n = 39$, $P < 0.01$). The patients with fever or weight loss had higher blood levels of IL-6. IL-6 blood levels were also higher in patients with lymph node invasion and/or distant metastases (94.7 pg/ml, 39.0–150.4, $n = 15$) than in patients with undisseminated RCC (7.4, 4.1–10.7, $n = 63$, $P < 0.0001$). An abnormal IL-6 plasma value (>40 pg/ml) had a positive predictive value of 91.0% for lymph node and/or metastatic spread of RCC. IL-6 was statistically correlated with C-reactive protein (nephelometric assay) blood values ($r' = 0.67$, $n = 78$, $P < 0.001$). The TNF-α and IL-1β levels were not significantly different in patients with or without fever or weight loss. The plasma levels of the three cytokines were not correlated with the size of the primary tumour. An increased plasma value of IL-6 is a good marker for tumour dissemination in patients with untreated RCC.
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INTRODUCTION

RENAL CELL carcinoma arises from the renal tubular epithelial cells, and is the most common malignant disease of the kidney. The prognostic indicators currently used in renal cell carcinoma are mainly local extension and metastatic spread of the tumour with the presence or absence of metastatic disease at the time of surgery being the main factor in determining survival. When renal cell carcinoma is not cured by surgery, it remains one of the most therapy-resistant malignancies in humans [1–3]. There is a need for markers to enhance the ability to predict the clinical course of the disease, and to identify patients who may benefit from surgery.

Interleukin (IL)-1, tumour necrosis factor (TNF) and IL-6 are cytokines with overlapping biological properties which form a complex network of interactive signals. They are considered major mediators of fever [4, 5] and of the production of acute phase proteins [6]. Progressive tumours have several consequences on host physiology, particularly on the cytokine network. Different types of neoplastic cells have been shown to produce cytokines such as TNF [7] and IL-6 [8] and to be capable of inducing their production by stromal cells [8, 9]. Tumour cell and host-derived cytokines have been suggested to be responsible for cachexia associated with cancer [10, 11]. Alternatively, TNF, IL-1 and IL-6, by their biological properties, may alter growth and differentiation of neoplastic cells [12], including cells from renal cell carcinoma [13, 14].

Patients with renal cell carcinoma can exhibit systemic manifestations such as fever, weight loss and elevation of acute phase proteins [1]. The relationship with cytokine production has not yet been clearly established in these settings. We have measured IL-1β, TNF-α and IL-6 blood concentrations in a group of 78 consecutive patients with untreated renal cell carcinoma. The possible relationship between cytokine circulating levels and the characteristics of the patients was analysed.

PATIENTS AND METHODS

Patients and controls

Patients. This study was conducted between January 1990 and September 1992, according to the principles of the Declaration of Helsinki and the regulations of our institution. 78 consecutive patients with untreated renal cell carcinoma, admitted to the Department of Urology of La Pitié-Salpétrière Hospital or Saint-Antoine Hospital, Paris, France, and 56 healthy subjects were studied. Patients with a renal tumour different from renal cell carcinoma or with an associated disease were excluded.

63 of the patients with renal cell carcinoma were males and 15

Correspondence to C. Dosquet.
C. Dosquet and J.-L. Wautier are at the Laboratoire d'Immuno-Hématologie, Hôpital Lariboisière (Université Paris VII), 2, rue Ambroise Paré, 75010 Paris; A. Schaetz and F. Richard are at the Service d'Urologie; C. Faucher is at the Service de Néphrologie, CHU Pitié-Salpétrière (Université Paris VI), 83, Boulevard de l'Hôpital, 75013 Paris; E. Lepage is at the Département de Biostatistique et d'Informatique, Hôpital Saint-Louis (Université Paris VII), 1 Avenue Claude-Vellefaux, 75010 Paris; and J. Cabane is at the Département de Médecine Interne, CHU Saint-Antoine (Université Paris VI), 184 Faubourg Saint-Antoine, 75012 Paris, France.
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were females. Their ages ranged from 29 to 87 years (61.1 ± 12.2, mean ± S.D.) 10 patients (13%) had prolonged non-infectious fever (higher than 38°C for more than 1 week), and 11 patients (14%) had weight loss (10% or higher in the last 6 months). Before surgery, all patients were subjected to clinical and chest X-ray examinations, bone scan, abdominal ultrasonography and abdominal computerised tomography. In 9 patients, distant metastases were diagnosed before surgery, and 2 of them underwent surgery for a renal cell carcinoma associated with a single metastasis. For 3 other patients, the distant metastases were discovered during the month following nephrectomy. Staging was done according to the TNM classification [15] modified for T3 (T3a = tumour invades perinephric tissues but not beyond Gerota's fascia, T3b = tumour extends into renal vein(s), T3c = tumour extends to vena cava; T4 = patients with homolateral invasion of adrenal gland) [2]. Clinical preoperative and histopathological findings were collected for staging at the time of the operation. T and N were always defined as pT and pN. The operated patients had a nephrectomy (radical $n = 67$, partial $n = 2$, enucleation $n = 2$) and a dissection of the lymph nodes of the latero-aortacaval area. The patients with lymph node invasion were staged as N+. M was defined according to the clinical findings if no histopathological data on distant metastases were available. The 3 patients diagnosed as metastatic after surgery were staged as M+ for the purpose of this analysis. The TNM staging of the 71 patients who underwent surgery is presented in Table 1.

Healthy subjects. Normal healthy subjects were recruited among blood donors ($n = 56$, 27 females, 29 males; mean age 41.5 years, range 21–65), and formed a control group to establish the normal blood levels of cytokines and C-reactive protein (CRP). The absence of disease was assessed by clinical examination and routine laboratory tests, including peripheral blood cell count, screening for anti-human immunodeficiency virus, anti-hepatitis B core antigen and anti-hepatitis C virus antibodies, hepatitis B surface antigen, serum aminotransferase (ALT) and serological tests for syphilis.

Blood collection. For each patient, the day before treatment, and each healthy subject venous blood was collected into dry or EDTA-coated sterile tubes. Serum and plasma were separated and frozen (−80°C) immediately in aliquots of 0.5 ml until tests were performed.

Methods

Cytokine assays. The plasma levels of IL-1β and TNF-α were determined by an immunoradiometric method (IL-1β and TNF-α, IRMA, Medgenix, Fleurus, Belgium). IL-6 plasma levels were assayed using a sandwich enzyme immunoassay technique (IL-6 EASIA, Medgenix). The minimum detectable concentrations are estimated to be 5 pg/ml for IL-1β and TNF-α and 3 pg/ml for IL-6. The specificity of the three immunoassays has been tested with standards together with different cytokines at a concentration of 500 ng/ml. IL-1β immunoassay did not cross-react (<0.01%) with IL-1α, IL-2, IL-3, IL-4, IL-6, IL-8, TNF-α, TNF-β, GM-CSF (granulocyte–macrophage colony-stimulating factor) or interferons α, β and γ. The TNF-α immunoassay did not cross-react (<0.005%) with TNF-β, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, GM-CSF or interferons α, β and γ. The IL-6 immunoassay did not cross-react (<0.01%) with IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-7, IL-8, TNF-α, TNF-β, GM-CSF or interferons α, β and γ.

The inter- and intra-assay variations of these techniques were all less than 10%. Each sample was tested in duplicate. The normal plasma values were for IL-1β: 5.4 pg/ml [mean, 95% confidence interval (CI) 4.0–6.8, $n = 44$], for TNF-α: 3.0 pg/ml (95% CI 2.1–3.9, $n = 56$), and for IL-6: 11.6 pg/ml (95% CI 10.1–13.1, $n = 39$).

CRP assay. CRP was measured in serum samples by immunonephelometric assay (Behringwerke nephelometer analyser; Behringwerke AG, Marburg, Germany) using an appropriate antiserum (NA latex CRP reagents). Using this technique, the lower limit of detection was 2.5 mg/l and the normal serum values of CRP were found to be less than 9 mg/l (mean + 2 S.D., $n = 38$).

Statistical analysis. The values for cytokine blood levels in the different groups are given as mean and 95% CI. The figures show the individual values (mean of two measurements) of each parameter. The differences between the cytokine plasma levels of the patients with renal cancer and healthy subjects were analysed using the non-parametric Mann–Witney rank sum test. The non-parametric Kruskal–Wallis test was used to evaluate the relationship between cytokine blood levels and the clinical and staging characteristics of the renal cancer patients. Correlation coefficients between the different parameters were calculated by the non-parametric Spearman rank test. Statistical analysis was performed using the BMDP statistical software package (BMDP statistical software, Los Angeles, California, U.S.A., 1990).

Table 1. TNM staging of the 71 patients with renal cell carcinoma who underwent surgery

	T1	T2	T3a	T3b	T3c	T4
n	7	47	6	5	1	5
N−M−	7	45	4	4	1	2
N+M−	0	0	1	0	0	2
N−M+	0	2	0	0	0	0
N+M+	0	0	1	1	0	1

The details for the staging are given in the Patients and Methods section.

RESULTS

Plasma cytokines in patients with renal cell carcinoma

Of the group of 78 patients with renal cell carcinoma, IL-1β was measured in 60 consecutive patients (8.5 pg/ml, 95% CI 5.9–11.1), and was similar to IL-β levels in healthy subjects (5.4 pg/ml, 95% CI 4.0–6.8, $n = 44$, $P = 0.76$) (Fig. 1a). The proportion of patients with distant metastases was the same in this group of 60 patients and in the 78 patients.

Blood TNF-α levels in patients with renal cancer (9.8 pg/ml, 95% CI 7.8–11.8, $n = 78$) were low, but significantly higher than TNF-α levels in healthy subjects (3.0 pg/ml, 95% CI 2.1–3.9, $n = 56$, $P < 0.0001$, Fig. 1b). In patients with renal cell carcinoma, some patients were older than their corresponding normal controls. An influence of age on TNF and IL-6 production has been reported [16], for this reason the correlation coefficient between the age of cancer patients and their TNF-α blood levels was calculated ($r' = 0.37$, $n = 78$, $P < 0.01$). The TNF-α blood values found in patients older than 65 years (13.6 pg/ml, 95% CI 9.9–17.3, $n = 32$) were statistically higher than the TNF-α values found in patients younger than 65 years (7.2 pg/ml, 95% CI 5.4–9.0, $n = 46$, $P < 0.01$). The number of patients with

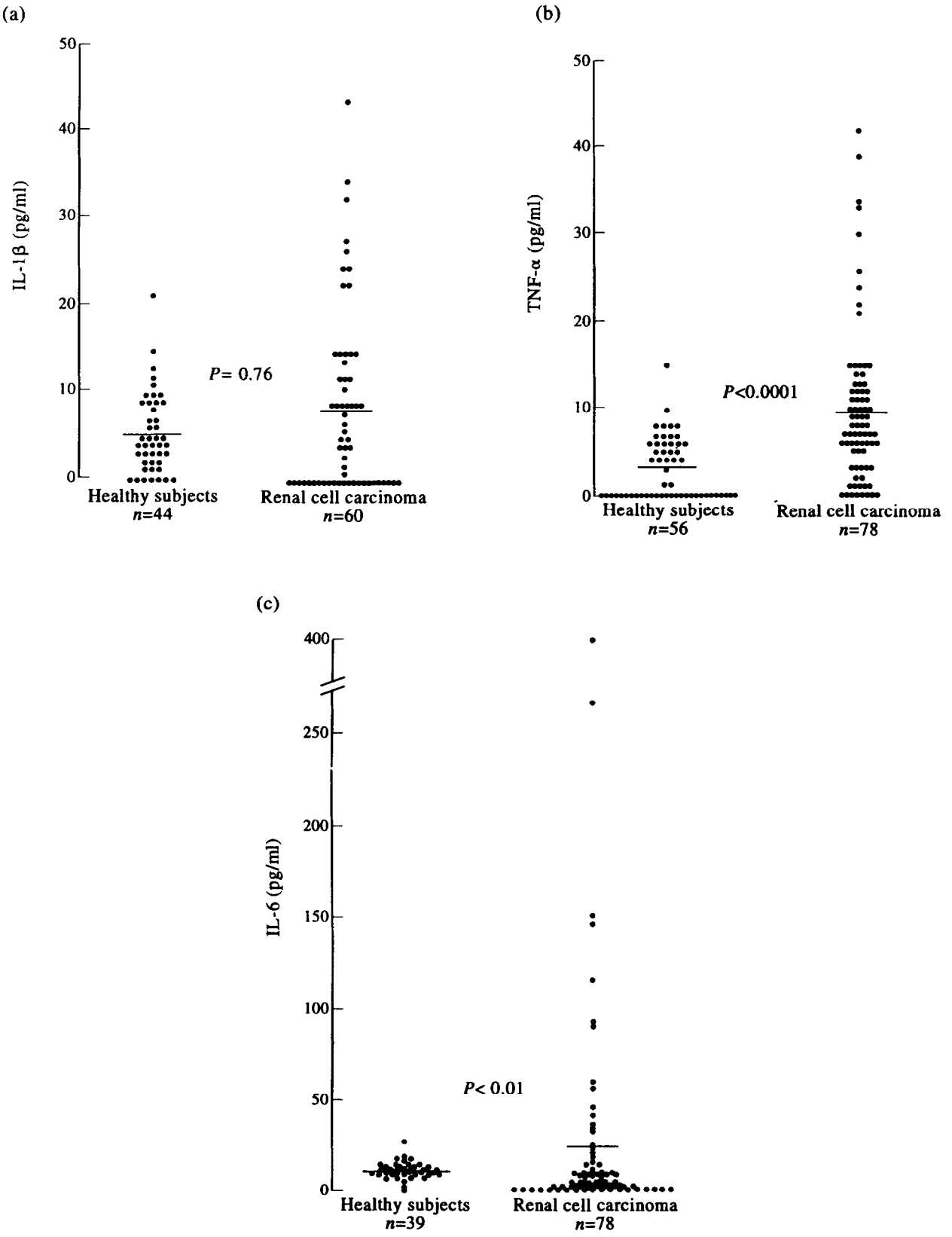

Fig. 1. Individual plasma values (mean of two determinations) of cytokines, (a) IL-1β; (b) TNF-α; (c) IL-6 in healthy subjects and in patients with renal cell carcinoma. Bars denotes the mean values.

distant metastases ($n = 6$) was the same in each group. Age did not account for the higher TNF since a statistically significant difference still remained when the TNF-α blood concentrations of younger patients ($n = 46$) were compared with those measured in normal subjects of the same age ($n = 56, P < 0.001$).

The IL-6 plasma concentrations in patients with renal cancer (24.2 pg/ml, 95% CI 11.1–37.3, $n = 78$) were statistically higher than the IL-6 concentrations in healthy subjects (11.6 pg/ml, 95% CI 10.1–13.1, $n = 39, P < 0.01$, Fig. 1c). 14 patients with untreated renal cell carcinoma had IL-6 blood concentrations

outside the range of normal values. No statistically significant correlation ($r' = 0.04$, $n = 78$) was found between age and the IL-6 plasma levels measured in patients with renal cell carcinoma.

Analysis of the relationship between cytokine blood levels and the characteristics of renal cancer patients

The concentrations of TNF-α in the blood of patients with renal cell carcinoma were similar regardless of whether fever or weight loss was present. But the patients with fever or weight loss had higher blood levels of IL-6 (Table 2). In patients with disseminated renal cell carcinoma (i.e. with distant metastases, $n = 12$, or with lymph node invasion and/or distant metastases, $n = 15$), the TNF-α plasma values were slightly but significantly increased (14.3 pg/ml, 95% CI 9.4–19.2, $n = 15$) compared with patients with undisseminated renal cancer (8.7 pg/ml, 95% CI 6.5–10.9, $n = 63$, $P < 0.05$, Fig. 2a). IL-6 blood values in patients with disseminated renal cell carcinoma (94.7 pg/ml, 95% CI 39.0–150.4, $n = 15$) were dramatically increased in comparison with IL-6 blood concentrations in patients with undisseminated cancer (7.4 pg/ml, 95% CI 4.1–10.7, $n = 63$, $P < 0.0001$, Fig. 2b). 2 of the 3 patients without metastasis but with lymph node invasion had abnormal IL-6 (42 and 145 pg/ml). The plasma levels of TNF-α and IL-6 were not correlated with the size of the primary tumour.

An abnormal IL-6 plasma value (>30 pg/ml) had a positive predictive value for lymph node invasion and/or distant metastases of 71.4% (sensitivity 66.7%, specificity 93.7%) [17]. When patients with plasma IL-6 above 40 pg/ml were considered, the positive predictive value was 91.0% (sensitivity 66.7%, specificity 98.4%).

IL-1β plasma levels were not correlated with the characteristics of the patients.

Correlation of IL-6 and CRP blood levels in patients with renal cell carcinoma

No statistically significant correlation was found between the blood levels of TNF-α and IL-6 ($r' = 0.15$, $n = 78$), or between the blood levels of TNF-α and CRP ($r' = 0.17$, $n = 78$) in patients with untreated renal cell carcinoma. However, the CRP blood concentration was correlated with the IL-6 blood concentration ($r' = 0.67$, $n = 78$, $P < 0.001$, Fig. 3).

DISCUSSION

In this study, we observed that IL-1β blood concentrations were similar in patients with renal cell carcinoma and in healthy subjects, but that circulating TNF-α was slightly augmented and IL-6 significantly increased in patients. It has previously been reported that in pathological conditions TNF-α and IL-6 could be detected in the blood in the absence of detectable IL-1 [5, 18]. A method including an extraction step has been proposed to improve the sensitivity of IL-1 plasma determination [19].

Using a sensitive immunoradiometric assay [20], we found increased TNF-α plasma levels in patients with renal cell carcinoma. Conflicting results have been reported concerning the presence of TNF in the blood of patients with cancer [21, 22], and no correlation has been found between circulating TNF levels and clinical cancer cachexia [21, 23]. A systemic release of TNF-α, together with IL-6, has been shown to coincide with an increase in body temperature, but these observations concerned lipopolysaccharide-induced fever. [5]. In metastatic renal cell carcinoma treated with interferon γ, the changes in TNF-α serum levels did not correlate with the increase in temperature [24]. Waase and colleagues reported that TNF mRNA was expressed in infiltrating monocytes/macrophages in renal cell carcinoma, but never by the tumour cells. Their study suggested that the degree of macrophage activation and TNF production depends on tumour spread to the draining lymph nodes [25].

We have found that increased plasma levels of IL-6 correlated with the presence of fever in our group of renal cancer patients, as reported recently [26]. Blood concentrations of IL-6 were increased in our patients with renal cell carcinoma and weight loss. Systemic IL-6 could play a role in the development of cancer cachexia as suggested by animal models [11]. The presence of systemic IL-6 has been reported in animals implanted with tumour cells [18], as well as in humans with cancer [27]. It is now well established that tumour cells from renal cell carcinoma can produce IL-6 [13, 14, 26, 28] and express IL-6 receptors [28]. Blay and colleagues have reported that IL-6 was above the normal level in patients with renal cell carcinoma and distant metastases [29]. Here we show that plasma IL-6 is a good marker for metastases in patients with renal cell carcinoma. The mechanism responsible for the increased IL-6 level remains unclear. It could be due either to the abnormal production by the tumour cells or to the response of the immune system. A correlation between the tumour burden and circulating IL-6 has been observed in animal experiments [30] and in clinical studies [27]. It has been suggested that the level of IL-6 production by renal cell carcinoma could be involved in the progression of the tumour *in vivo* [28, 29]. In our study, as observed previously by others, the CRP serum concentration correlated with plasma IL-6 concentration [29], and could also be a good marker for disease severity.

In this study of renal cell carcinoma, we have observed that IL-6 could be a good marker for a metastatic spread of the tumour. In the absence of clinical evidence of dissemination, an

Table 2. TNF-α and IL-6 blood values (mean and 95% CI) in renal cell carcinoma patients according to the presence or absence of fever (above 38°C) or weight loss (>10% ideal weight)

	Symptoms			
	Fever (n=10)	Apyrexia (n=68)	Weight loss (n=11)	Normal weight (n=67)
TNF-α (pg/ml) (95% CI)	14.7 (8.3–21.1)	9.1 (7.1–11.1)	12.6 (6.7–18.5)	9.4 (7.3–11.5)
IL-6 (pg/ml) (95% CI)	51.0 (21.3–80.7)	20.3* (13.0–27.6)	86.6 (19.7–153.5)	13.9** (4.8–23.0)

*$P<0.01$, **$P<0.001$.

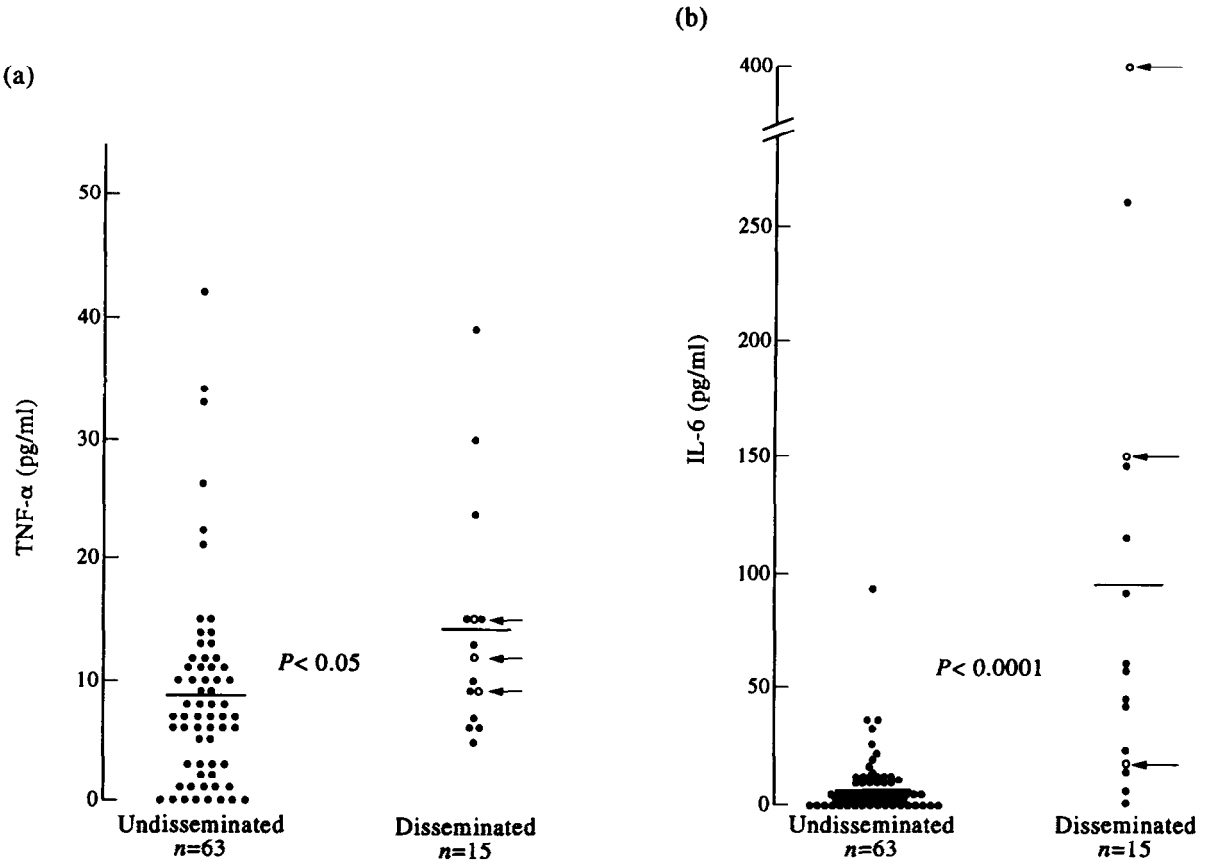

Fig. 2. Individual plasma values of (a) TNF-α and (b) IL-6 in patients with undisseminated or disseminated (lymph node invasion and/or distant metastases) renal cancer. The open circles represent the 3 patients diagnosed as metastatic after surgery. Bars denote the mean values.

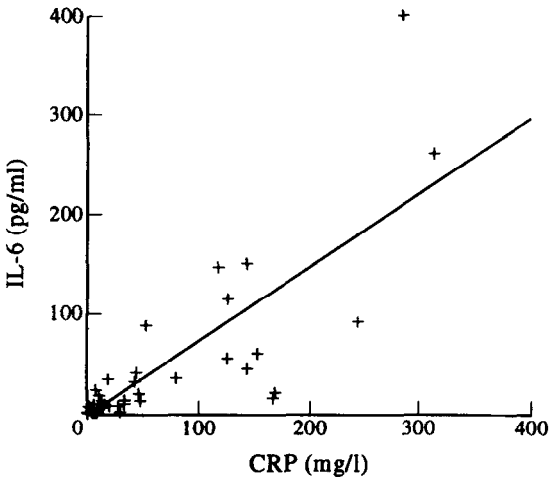

Fig. 3. The correlation between blood values of IL-6 and C-reactive protein (CRP) in the 78 patients with renal cell carcinoma was statistically significant (Spearman's rank test) ($r' = 0.67, P < 0.001$).

increased initial concentration of IL-6 can support a more extensive assessment in patients with renal cell carcinoma.

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Low-dose Interleukin-2 Subcutaneous Immunotherapy in Association with the Pineal Hormone Melatonin as a First-line Therapy in Locally Advanced or Metastatic Hepatocellular Carcinoma

R. Aldeghi, P. Lissoni, S. Barni, A. Ardizzoia, G. Tancini, A. Piperno, M. Pozzi, G. Ricci, A. Conti and G.J. M. Maestroni

Experimental studies have showed that hepatocellular carcinoma (HCC) cells are susceptible to cytolysis of interleukin (IL)-2-activated lymphocytes. Moreover, our previous studies demonstrated that the pineal neurohormone melatonin (MLT) may enhance IL-2 efficacy. On this basis, a study was started with low-dose IL-2 (3 million U/day subcutaneously for 6 days/week for 4 weeks) plus MLT (50 mg/day orally every day given in the evening) as a first-line therapy of unresectable HCC. The study included 14 patients. Objective tumour regressions were obtained in 5/14 (36%) patients (one complete response, four partial responses), with a median duration of 7+ months. 6 patients had stable disease, while the other 3 progressed. Toxicity was low in all cases. This study shows that the neuroimmunotherapy with low-dose IL-2 plus MLT is a new well-tolerated and effective therapy of advanced HCC.

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