Pergamon

European Journal of Cancer Vol. 33, No. 11, pp. 1794–1798, 1997

Printed in Great Britain
0959-8049/97 $17.00 + 0.00

PII: S0959-8049(97)00179-2

Original Paper

Serum Interleukin-6 in Relation to Acute-phase Reactants and Survival in Patients with Renal Cell Carcinoma

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Patients with malignancies often present with signs of inflammatory reactions such as elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Since interleukin-6 (IL-6) is a possible regulator of these reactions and has been proposed as a predictor of prognosis, the aim of the study was to analyse its clinical significance in patients with renal cell carcinoma. Serum samples were collected from 196 patients before any treatment. IL-6 was analysed by an enzyme-linked immunoassay and compared with tumour grade, stage, acute-phase reactants and survival. Patients with renal cell carcinoma had significantly higher IL-6 levels (mean 28.1 ± 63.4 ng/l; median 8.3 ng/l) compared with controls (mean 1.7 ± 2.6 ng/l; median 0.5 ng/l; $P < 0.001$). Serum IL-6 levels in patients with distant metastases were significantly higher than for patients with tumours confined to the kidney ($P = 0.02$). This difference was more pronounced when serum IL-6 levels in patients with poorly differentiated tumours were compared with well-differentiated tumours ($P < 0.001$). A significant correlation between the acute-phase reactants CRP, ESR and IL-6 levels was found. Survival time was significantly shorter ($P = 0.001$) for patients with IL-6 levels above the median serum level compared with patients with lower levels. Similar significant prognostic results were obtained in the group of patients with metastatic disease, but not in group of patients with stage I–III. Serum levels of IL-6 correlated to tumour stage, grade and acute-phase reactants. Increased levels were related to the presence of metastases and adverse survival. Serum IL-6 proved univariate prognostic information but this prognostic significance was lost using a multivariate analysis. © **1997 Elsevier Science Ltd.**

Key words: renal cell carcinoma, interleukin-6, tumour stage, erythrocyte sedimentation rate, CRP, prognosis

Eur J Cancer, Vol. 33, No. 11, pp. 1794–1798, 1997

INTRODUCTION

RENAL CELL carcinoma is the most common malignancy of the kidney. Prognosis of the disease is mainly dependent on the local extension and the metastatic spread of tumour [1, 2]. Approximately a third of the patients already have metastatic disease at the time of diagnosis and approximately 50% of the remaining patients will display distant metastases during the follow-up. Metastatic renal cell carcinoma remains one of the most therapy-resistant malignancies. Despite promising results with some immunotherapeutics, tumour responses are infrequent and when they occur are most often partial and of short duration [3]. There is a clinical need for improving the ability to predict prognosis for individual patients with renal cell carcinoma in order to select those patients who are at risk of tumour progression and also those patients that might respond to different modes of therapy and achieve a prolonged survival.

Interleukin-6 (IL-6) is a multifunctional cytokine which plays a central role in the host defence mechanism by regulating immunoresponses, haematopoiesis, and acute-phase reactions [4, 5]. Furthermore, IL-6 regulates the proliferation and differentiation of immunocompetent, natural killer and cytotoxic cells and normal haematopoietic progenitors [6, 7]. It is also a potent pro-inflammatory cytokine. IL-6

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Received 18 Dec. 1996; revised 17 Mar. 1997; accepted 26 Mar. 1997.

acts as an endogenous pyrogen and induces the expression of acute phase protein genes including the C-reaction protein (CRP) gene [8]. It has been reported that the serum IL-6 level might be an adverse prognostic factor in patients with renal cell carcinoma [9–11]. Due to the limited knowledge of the role IL-6 in patients with renal cell carcinoma, the aim of the present study was to compare serum levels of IL-6 with clinical outcome, based on survival of the patients and tumour stage, grade, tumour size, and the acute-phase reactants and erythrocyte sedimentation rate (ESR).

PATIENTS AND METHODS

Patients

A total of 196 patients with histologically verified renal cell carcinoma, admitted to the department of Urology and Andrology, University Hospital, Umeå, were included in the study. Serum samples were obtained before any treatment after informed consent of the patients. There were 119 men and 77 women, with a mean age of 64.3 years, ranging from 25 to 86 years. Seven patients received palliative treatment only, due to advanced metastatic spread and the remaining 189 patients were surgically treated, 186 with radical nephrectomy and 3 with partial resection. Radical retroperitoneal lymph node dissection was not routinely performed. Pre-operatively, the patients were examined with chest x-ray, ultrasound and computerised tomography (CT) and the patients with symptoms or signs underwent bone scans, magnetic resonance imaging (MRI) and cavographies. The patients were staged according to the TNM classification system [12]. Specimens were histopathologically graded according to a four-graded scale of Skinner and associates [2]. The patients were followed according to scheduled follow-up programme including clinical and radiological examinations. In April 1996, the mean follow-up time for surviving patients was 79 months (median 74, range 14–154 months). Sera from 24 patients with benign kidney masses (e.g. 19 simple renal cysts, 2 oncocytomas, 1 angiomyolipoma and in 2 patients columna Bertini) were used as clinical control patients.

Methods

Serum samples were collected before initiation of any therapy between 8 and 11 a.m., and stored at −80°C until analysis at the department of Clinical Chemistry. Serum IL-6 concentration was quantified using an enzyme-linked immunosorbent assay (Endogen, Cambridge, Massachusetts, U.S.A.). Values above 400 ng/l were set at 400 ng/l and values below 1 ng/l were set at 0.5 ng/l due to the limitations of the standard curve. A standard curve was run for each plate of the kit. Each serum sample was analysed in duplicate. The manufacturer reports normal mean values of 0.7 ng/l (range 0–5 ng/l). Serum CRP was quantified by rate nephelometry using a Beckman Array™ protein instrument. Calibrators were from Beckman Instruments Inc., Fullerton, California, U.S.A. The ESR was determined according to the Westergren method. The reference values of the acute-phase reactants according to the department of Clinical Chemistry were: CRP <10 mg/l and ESR <28 mm/h.

Statistics

Statistical analysis was performed using Fisher's exact probability test, Mann–Whitney U-test and the survival curves were calculated according to the method of Kaplan–Meier. Survival time from admission was compared using the log rank test. The multivariate analysis was performed according to the Cox regression hazard model.

RESULTS

Patients with renal cell carcinoma had significantly ($P < 0.001$) higher IL-6 levels (mean 28.1 ± 68.4 ng/l; median 8.3 ng/l) than control patients (mean 1.7 ± 2.6 ng/l and median 0.5 ng/l). A significant correlation between IL-6 levels and both tumour stage and grade was observed (Table 1). There was no correlation between IL-6 and age or gender of the patients, nor was there any correlation between IL-6 and tumour size (data not shown).

Significant linear correlations between IL-6 and the acute-phase reactants CRP and ESR were demonstrated ($r = 0.22$, $P = 0.005$, and $r = 0.18$, $P = 0.015$, respectively). When CRP and ESR were analysed in relation to three levels of IL-6 (<1.0, 1.0–20, and >20 ng/l), significantly increased serum levels were found with increasing serum IL-6 levels (Table 2).

At the end of the follow-up period, 66 patients were alive, 102 had died of the disease and 28 patients had died of intercurrent diseases. For all patients, there was a significant survival advantage for patients with IL-6 values below the median (8.3 ng/l) compared with those with levels above the median value ($P = 0.001$, Figure 1). Such a survival advan-

Table 1. Serum interleukin-6 levels in relation to tumor stage and grade in 196 patients with renal cell carcinoma

		Interleukin-6 level (ng/l)		
	No	Mean ± S.D.	Median	Range
Total stage (TNM)	196	28.1 ± 63.4	8.3	0.5–400
I–II	76	26.1 ± 62.8	3.0	0.5–400
			NS	
III	54	20.8 ± 34.4	9.7	0.5–181
			NS	
IV	66	36.4 ± 80.2	11.5	0.5–400
Grade				
1–2	45	20.0 ± 45.3	2.7	0.5–276
			NS	
3	102	24.0 ± 61.3	6.8	0.5–400
			$P < 0.001$	
4	49	44.2 ± 78.7	21.3	0.5–400

$P = 0.02$ (I–II vs III); $P = 0.001$ (1–2 vs 3)

Table 2. CRP and ESR levels in relation to levels of serum interleukin-6 concentration in 196 patients with renal cell carcinoma

Reactant (mean ± S.D.)	Interleukin-6 level (ng/l) <1.0	1.0–20.0	>20.0
		<0.001	
	<0.001		=0.006
CRP (mg/l)	9.1 ± 9.9	35.1 ± 43.9	66.8 ± 51.6
range	1–40	0–212	1–174
		<0.001	
	<0.001		=0.001
ESR (mm/h)	27.7 ± 22.1	52.0 ± 34.7	72.1 ± 43.4
range	2–75	2–150	5–139

tage was also shown for patients with distant metastatic disease (stage IV) ($P = 0.003$), but not for patients with stage I–III disease.

Multivariate analysis

With a stepwise elimination multivariate analysis, tumour stage, grade and ESR were shown to provide independent prognostic information, while IL-6 and the other factors lost their prognostic significance (Table 3).

DISCUSSION

The present study shows that serum IL-6 levels were significantly elevated in patients with renal cell carcinoma compared with control patients. Our controls with benign expansive renal masses had serum levels of the same magnitude as previously reported in healthy normal volunteers and control patients [13–15]. There was a correlation between elevated serum IL-6 levels and the occurrence of metastatic spread and adverse survival. In our study, all serum samples were taken before lunch, minimising the effects of the circadian fluctuation of IL-6 [13]. In other studies, elevated levels of IL-6 correlated with adverse prognosis in lymphoma and lung and ovarian carcinoma [14–16]. In studies on renal cell carcinoma, Blay and associates [9] have shown that the time interval between the diagnosis of primary tumours and metastases was shorter in patients with detectable serum IL-6 levels at diagnosis. Dosquet and associates [10] found that higher IL-6 serum levels were correlated with lymph node invasion and distant metastases. Serum IL-6 levels have also been shown to be a prognostic factor for response to IL-2 treatment. Patients with high IL-6 levels did not respond to IL-2 treatment [9]. Our findings confirm the results showing that increased IL-6 levels correlated with short survival and occurrence of metastases.

The increased production of IL-6 in renal cell carcinoma might be due to endocrine or exocrine tumour effects. Fresh renal carcinoma cells and cell lines have been reported to express IL-6 mMRA and to release IL-6 to the supernatant [17–19]. Others have shown that IL-6 levels are 10-fold higher in the renal vein compared to peripheral blood [20]. These results indicate that IL-6 is produced by the renal carcinoma cells and is released into the circulation. Our results are consistent with these observations and demonstrate that apart from IL-6 levels being increased in serum of patients with renal cell carcinoma, the serum levels are also increased in higher stage and grade.

Some reports support the hypothesis that IL-6 is also a growth factor for ovarian and renal cell carcinoma cells [17, 21]. In breast cancer, Tamm and associates [22] showed that exogenous IL-6 increases the motility and decreases adherence of breast cancer cell lines, suggesting that IL-6 may promote tumour metastasis and invasiveness *in vito*.

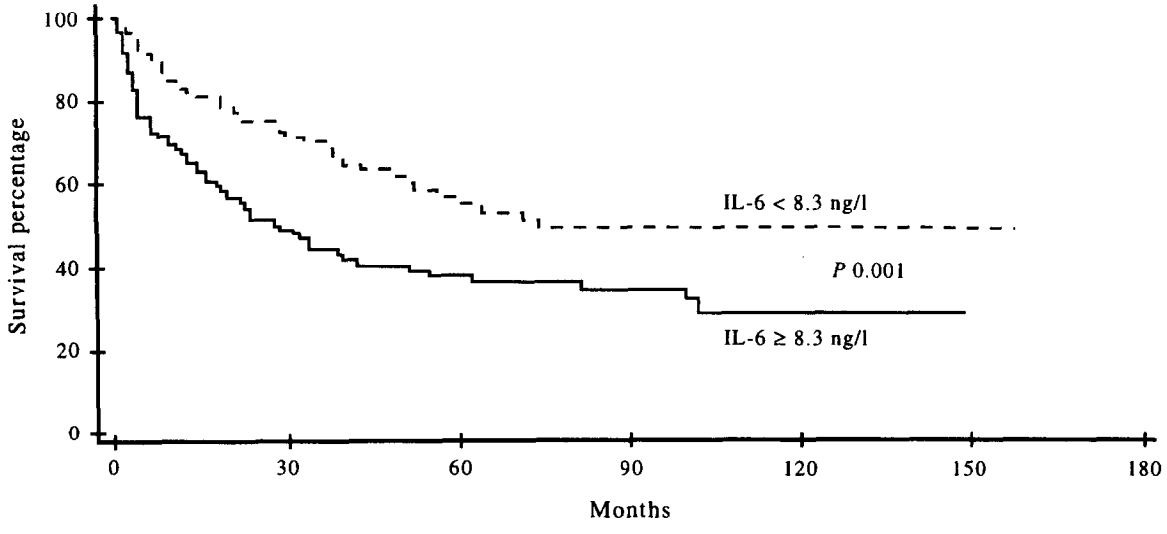

Figure 1. Kaplan–Meier survival curves showing survival time of 98 patients with renal cell carcinoma with IL-6 values below the median IL-6 value (<8.3 ng/l) compared with 98 patients with IL-6 values above or equal to the median value (≥8.3 ng/l).

Table 3. Multivariate analysis of prognostic factors analysed in a hazard regression model according to Cox, based on 194 patients with renal cell carcinoma

Prognostic factor	β value	EXP[β]	*P* value	95% confidence interval lower–upper
Age (<64 years versus ≥64 years)	0.368	1.444	0.083	0.954–3.014
Sex (male versus female)	0.328	1.387	0.132	0.904–2.127
Grade (1–2 versus 3–4)	1.644	5.175	0.0005	2.064–12.973
Stage (I–II versus III–IV)	2.332	10.302	0.0000	6.467–16.409
CRP	0.357	1.430	0.173	0.855–2.391
ESR	0.494	1.638	0.056	0.987–2.721
IL-6	0.001	1.001	0.580	0.998–1.004
Final stepwise analysis model				
ESR	0.623	1.864	0.002	1.248–2.785
Grade	1.711	5.550	0.0003	2.216–13.903
Stage	2.136	8.469	0.0000	5.499–13.043

Alternatively, IL-6 could exert an adverse effect through modulation of the antitumour response. Renal carcinoma cells also express the IL-6 receptor m-RNA [17], suggesting that this cytokine might function as an autocrine regulator. In ovarian cancer, increased IL-6 serum levels were accompanied by augmented IL-6-receptor concentrations [15].

Interleukin-6 is an important factor in the immune and haematopoietic systems and is the major mediator of the hepatic acute-phase response. It is an endogenous pyrogen and induces the production of acute-phase proteins including CRP [4, 5]. It has previously been shown that serum CRP levels correlate with serum IL-6 levels in patients with renal cell carcinoma [9]. Our data are consistent with these observations and show that serum CRP and ESR levels were significantly correlated with serum IL-6 levels, data that might suggest that IL-6 is involved in the inflammatory response of the tumour. Both CRP and ESR has been shown to predict prognosis in renal cell carcinoma [23]. In the present study, IL-6 also showed a prognostic correlation. However, by stratifying the patients, a prognostic significance could only be shown in patients with metastatic disease. In a multivariate analysis, this prognostic significance was lost, while ESR and also tumour stage and grade were independent prognostic factors, in line with the results obtained in a previous study of other acute-phase reactant proteins [23]. Thus, in patients with renal cell carcinoma, ESR seems to be a more useful prognostic predictor than IL-6.

The reason for the increase in IL-6 levels in patients with renal cell carcinoma might be multifunctional. Some results indicate that IL-6 is produced by the tumour itself while other studies show that IL-6 production might be exocrine due to a general inflammatory response induced by the tumour. It is accepted that IL-6 regulates the proliferation differentiation of immunocompetent cells such as natural killer and cytotoxic cells [6, 7]. The data in the study seem to be contradictory since increased serum IL-6 levels did not to correspond to antitumour effects, but rather indicated worse prognosis with progression of the tumour. Possibly the cytokine level is important for immunofunction, but might also be an effect of a pathological condition of the disease. Thus, the role of IL-6 in renal cell carcinoma remains unclear.

The present study shows that IL-6 provides prognostic information, but only in patients with IV disease. Increased levels correlated with the incidence of metastatic disease and adverse survival. Serum IL-6 values might help to monitor immune response and could be of value in the stratification for future therapy trials of metastatic renal cell carcinoma.

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Acknowledgements—This study was supported by the Swedish Cancer Society, Lions Cancer Research Foundation of Umeå University, The Medical Faculty, Umeå University and University Hospital, Umeå.