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Interleukin-6 Blood Level Is Associated With Circulating Carcinoembryonic Antigen and Prognosis in Patients With Colorectal Cancer

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Background: Interleukin-6 (IL-6) is an important proinflammatory cytokine that has multiple effects on stimulating inflammation and cell growth. Experimental data suggest that carcinoembryonic antigen (CEA) induces the systemic production of IL-6 and that IL-6 may stimulate tumor cell growth at metastatic sites. We tested the hypothesis that blood concentrations of IL-6 are associated with the amount of circulating CEA and with prognosis in patients with colorectal cancer.

Methods: CEA and IL-6 concentrations were measured by using enzyme immunoassay in preoperative serum samples from 208 patients with stages I through IV colorectal cancer.

Results: Linear regression analysis showed a significant association between serum values of CEA and IL-6 ($r = .544$; $R^2 = .296$; $P < .001$). Patients with stage III and stage IV disease had a significantly higher IL-6 serum concentration than those with stage I and stage II disease. In patients with stages I through III, 5-year survival was 83% in cases with concentrations of IL-6 at 10 pg/ml or less (n = 94) and 56% in cases with IL-6 concentrations of more than 10 pg/ml (n = 54; $P = .001$; median follow-up time, 46 months). By using multivariate analysis, an IL-6 concentration of more than 10 pg/ml was an independent prognostic factor of survival (relative risk = 1.820; $P = .020$).

Conclusions: In patients with colorectal cancer, blood concentration of IL-6 is associated with high circulating CEA and advanced stage. Furthermore, an IL-6 concentration of more than 10 pg/ml is an independent negative prognostic marker of survival.

Key Words: Colorectal carcinoma—CEA—Interleukin-6—Prognostic markers—Metastasis.

Carcinoembryonic antigen (CEA) is a highly glycosylated protein with a molecular mass of approximately 180 kDa. Originally described by Gold and Freeman[1] in 1965, it was the first tumor marker to be widely used for a common cancer. Serum concentrations of CEA are increased in patients with many types of cancer but especially those of the colon and rectum.[2]

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Elevated blood concentrations of CEA are associated with disease progression in patients with colorectal cancer (CRC).[3–5] However, the function of CEA in disease progression remains unclear.

Because CEA is a member of the immunoglobulin supergene family, it may be an adhesion molecule.[6] In vitro studies support this, because overexpression of CEA induces cells to aggregate with each other and CEA is the most important ligand for normal colonic epithelial cells.[7–9]

Systemic pretreatment of athymic nude mice with CEA increases the ability of weakly metastatic human CRC cells injected intrasplenically to colonize the liver with CRC cells that neither produce CEA nor bind to CEA in a solid phase in vitro.[10] As a result, CEA may have another function in metastasis, because it stimulates production of the cytokine interleukin (IL)-6 when in-

jected into mice.[11,12] IL-6 may be a potent stimulator of metastasis, because it upregulates the expression of adhesion receptors on endothelial cells as well as stimulates the production of growth factors.[13–17]

Therefore, CEA may promote hepatic metastasis by inducing IL-6 production, which, in turn, stimulates the expression of several adhesion receptors and growth factors that stimulate metastasis in the liver.

The purpose of this study was therefore to test the hypothesis that blood concentrations of IL-6 are associated with the amount of circulating CEA and with outcome in patients with CRC. Our approach was to measure the amount of CEA and IL-6 in the serum of 208 patients, who were explored at the University of Padova, for surgical management of CRC. The concentrations of CEA and IL-6 were then tested for their association with each other and with the clinical outcome.

MATERIALS AND METHODS

Serum Specimens and Patients

The preoperatively available stored sera of 208 patients, operated on for histologically confirmed colorectal adenocarcinoma at the Department of Surgery of the University of Padova, Italy, between 1991 and 1995, were used for this study. Cases with hereditary colorectal carcinoma, inflammatory bowel disease, and obstructing or perforated colorectal adenocarcinoma were excluded from the study. All patients underwent formal preoperative bowel preparation. Blood samples were obtained from fasting patients immediately before anesthesia and surgery. As a result, all patients had blood samples obtained at the same time point. After centrifugation at $3000 \times g$ for 10 minutes, sera were separated and stored at -80°C until the biochemical determinations.

The patient population consisted of 117 (56%) men and 91 (44%) women with a mean age of 64 years (range, 27–92 years). The tumor was located in the colon in 115 (55%) patients and in the rectum in 93 (45%). Histological grade was assessed according to World Health Organization criteria.[18] Fifty-two (25%) of the tumors were well differentiated, 134 (64%) moderately differentiated, and 22 (11%) poorly differentiated. According to the International Union Against Cancer classification and tumor, node, and metastasis staging system,[19] 54 (26%) of the tumors were stage I, 62 (30%) were stage II, 37 (18%) were stage III, and 55 (26%) were stage IV (of these, 12 patients underwent liver resection for hepatic metastases whereas the others had unresectable disease and underwent different forms of treatment). Patients with stage III colon cancer were given standard adjuvant chemotherapy (six cycles of 5-fluorouracil 375 mg/m^2 with leucovorin 100 mg/m^2/day for 5 days); patients with T3 or T4, or N1 or N2, rectal cancer received either preoperative chemoradiation therapy (n = 21; 4500 cGy/25 F, with concomitant 5-fluorouracil 375 mg/m^2 with leucovorin 10 mg/m^2/day for 5 days on days 1–5 and 29–33) or postoperative chemoradiation therapy (n = 16; 4500 cGy/25 F plus a 900 cGy/5 F boost, with concomitant 5-fluorouracil 375 mg/m^2 with leucovorin 10 mg/m^2/day for 5 days on days 1–5 and 29–33).

Assay of CEA and IL-6 Serum Concentration

Serum concentrations of CEA were measured by enzyme-linked immunosorbent assay by means of a method commercially available (Immuno One System; Bayer, Tarry Town, NY). Human IL-6 concentrations in sera were also determined by enzyme-linked immunosorbent assay, using a commercial kit (Endogen, Woburn, MA) according to the instructions of the manufacturer.

Statistical Analysis

Linear regression analysis was used to estimate the correlation between serum concentrations of CEA and IL-6. Kruskal-Wallis analysis of variance was used to assess the relationship between serum concentrations of CEA and IL-6 and different clinicopathological variables.

Survival curves were plotted by using the Kaplan-Meier method, and differences were assessed by the log-rank test.[20] The Cox proportional hazards model was used to determine the relative influence of the different covariates.[21] All statistical analyses were performed by using Statistica software (Statsoft, Tulsa, OK). $P < .05$ was considered significant.

RESULTS

Correlation Between CEA, IL-6, and Clinicopathological Variables

Elevated serum CEA (>5.0 μg/liter) was present in 35.6% of patients. Serum concentrations of CEA and IL-6 were not significantly associated with sex and age or tumor site and grade. On the other hand, serum concentrations of CEA were significantly higher in patients with more advanced tumor stage; patients with stage IV disease had a significantly higher serum concentration of CEA than those with stages I through III ($P < .01$), and patients with stage II and III disease had a significantly higher serum concentration of CEA than those with stage I ($P < .05$) (Fig. 1). A similar correlation was observed between serum concentration of IL-6 and tumor stage;

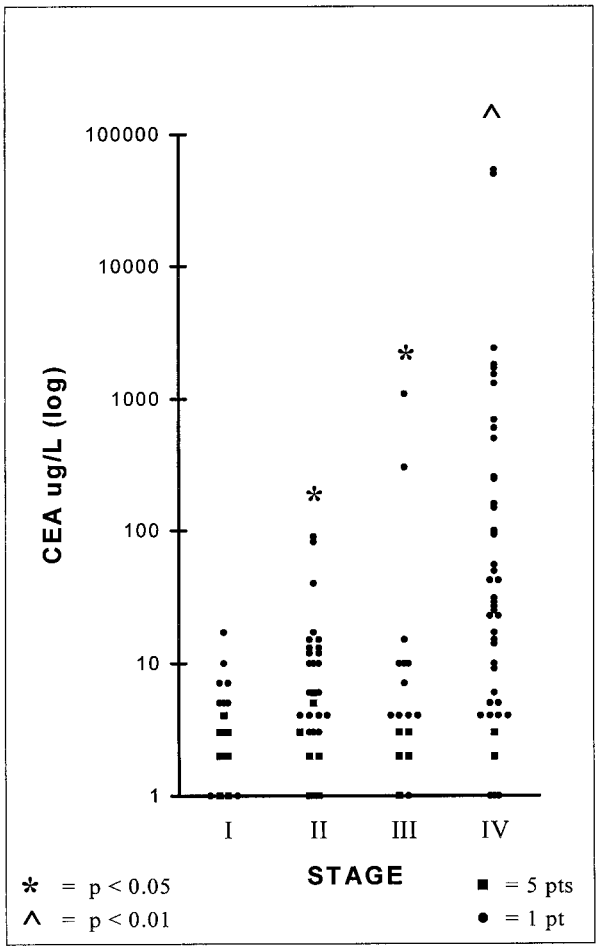

FIG. 1. Serum concentrations of carcinoembryonic antigen (CEA) in 208 patients with colorectal cancer according to tumor stage. Serum concentrations of CEA (μg/liter) are shown in a logarithmic scale for each tumor stage (I–IV).

patients with stage IV and III disease had a significantly higher serum concentration of IL-6 than those with stage I and II disease ($P < .05$) (Fig. 2).

Linear regression analysis showed a significant association between serum values of CEA and IL-6 ($r = .544$; $R^2 = .296$; $P < .001$). In the subset of patients with stage IV disease, serum concentrations of CEA and IL-6 were also correlated ($r = .596$; $R^2 = .355$; $P < .001$). Moreover, mean IL-6 serum concentration was 14.4 ± 2.1 pg/ml in the 134 patients with a normal CEA serum concentration (≤5.0 μg/liter) and 28.3 ± 7.5 μg/liter in the 74 patients with a high CEA serum concentration (>5.0 μg/liter; $P = .026$).

Serum Concentration of IL-6 and Prognosis

The analysis of survival was conducted on 198 of the 208 patients (there were two perioperative deaths and eight patients were lost from observation). During observation (median, 35 months), 70 patients died of disease (16% of stage I, 10% of stage II, 48% of stage III, and 77% of stage IV patients).

In Table 1, results of univariate analysis of survival in relation to clinicopathological findings are reported. The variables with a significant impact on survival were tumor grade, tumor stage, and serum concentrations of CEA and IL-6.

Kaplan-Meier survival curves, based on serum concentrations of IL-6 in the entire group of patients (stages I–IV), are shown in Fig. 3A. Patients with an IL-6 serum concentration of more than 10 pg/ml had a significantly shorter 5-year survival (45%) than patients with an IL-6 serum concentration of 10 pg/ml or less (69%; $P = .002$). As shown in Fig. 3B, a significant difference in

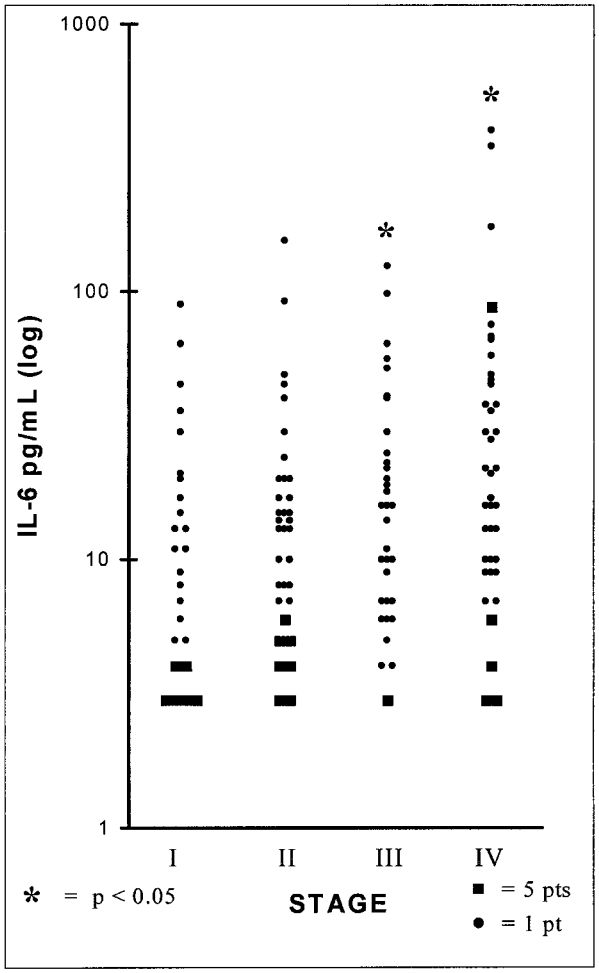

FIG. 2. Serum concentrations of interleukin-6 (IL-6) in 208 patients with colorectal cancer according to tumor stage. Serum concentrations of IL-6 (pg/ml) are shown in a logarithmic scale for each tumor stage (I–IV). Mean values are marked by bars.

TABLE 1. *Univariate analysis of survival in 198 patients with colorectal cancer*

Variable	Total	5-year survival (%)	Statistical significance
Sex			
Male	113	60	NS
Female	85	61	
Age			
<75 y	167	64	$P = .053$
≥75 y	31	44	
Tumor location			
Colon	107	64	NS
Rectum	91	57	
Tumor grade			
G1	50	67	
G2	125	62	$P = .013$
G3	23	37	
TNM stage			
I	51	87	
II	62	88	$P < .001$
III	35	33	
IV	50	19	
CEA			
≤5 μg/liter	132	66	$P < .001$
>5 μg/liter	66	49	
IL-6			
≤10 pg/ml	120	69	$P = .002$
>10 pg/ml	78	45	

NS, not significant; TNM, tumor, node, metastasis; CEA, carcinoembryonic antigen; IL-6, interleukin-6.

relation to serum concentration of IL-6 was also observed in the subset of patients with stages I through III disease. Five-year survival was 56% in patients with an IL-6 serum concentration of more than 10 pg/ml and 83% in patients with an IL-6 serum concentration of 10 pg/ml or less ($P < .001$).

Multivariate analysis of survival, using different factors such as sex (male vs. female), age (<75 years vs. ≥75 years), tumor site (rectum vs. colon), tumor grade (G1–G3), tumor, node, and metastasis stage (stages I–IV), CEA serum concentration (>5 μg/liter vs. ≤5 μg/liter), and IL-6 serum concentration (>10 pg/ml vs. ≤10 pg/ml), demonstrated that tumor stage, tumor site, and IL-6 serum concentration were independent prognostic factors of survival. Patients with an IL-6 serum concentration of more than 10 pg/ml had a relative risk of death of 1.820 ($P = .020$) (Table 2).

DISCUSSION

This study, conducted on 208 patients with CRC, demonstrated that preoperative serum concentration of IL-6 is associated with the amount of circulating CEA. Moreover, a high preoperative serum concentration of IL-6 was associated with advanced tumor stage and poor outcome.

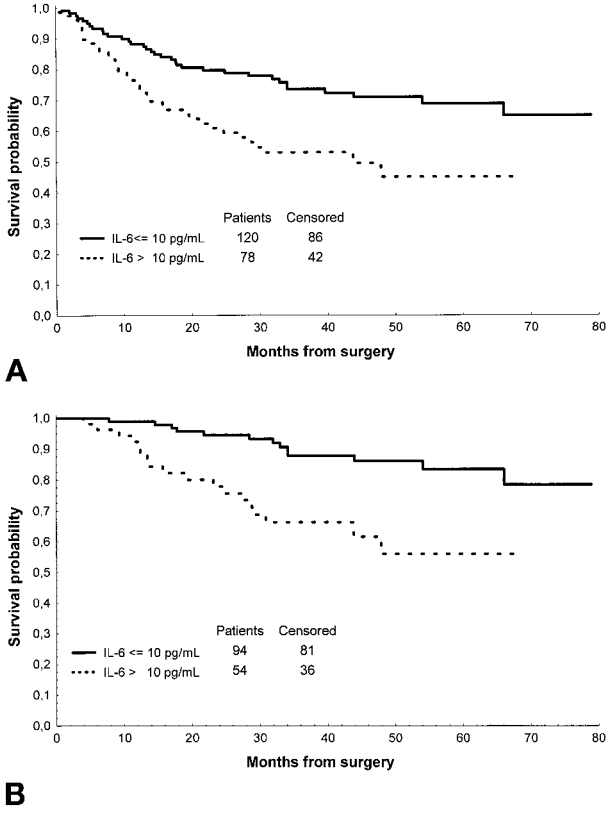

FIG. 3. Kaplan-Meier curves for overall survival in 198 patients with colorectal cancer, based on a comparison between low (≤10 pg/ml) and high (>10 pg/ml) serum concentration of interleukin-6 (IL-6). (**A**) Stages I through IV patients ($P = .002$). (**B**) Stages I through III patients ($P < .001$).

Experimental studies have recently demonstrated that in vitro treatment of Kupffer cells with CEA induces expression of cytokines such as IL-1α, IL-1β, IL-6, and tumor necrosis factor-α.[11] In addition, the injection of CEA into mice results in a significant dose-dependent IL-6 response.[12] These data may explain our finding of an association between concentrations of CEA and IL-6 in the serum of patients undergoing surgery for CRC. However, in the clinical setting, many confounding fac-

TABLE 2. *Multivariate analysis of survival in 198 patients with stages I–IV colorectal cancer*

Covariates	Relative risk	95% CI	*P*
Sex	.958	.637–1.708	.864
Age, y	1.432	.795–2.582	.231
Location	1.801	1.059–3.062	.029
Grade	1.133	.755–1.702	.544
TNM stage	2.760	2.103–3.622	<.001
CEA	1.630	.962–2.759	.068
IL-6	1.820	1.095–3.024	.020

CI, confidence interval; TNM, tumor, node, metastasis; CEA, carcinoembryonic antigen; IL-6, interleukin-6.

tors could be present; blood concentrations of IL-6 are, in fact, elevated in conditions such as sepsis, trauma, surgery, inflammatory bowel disease, and other forms of chronic stress.[22–25]

Goydos et al.[26] reported that the serum concentration of IL-6 did not correlate with CEA in a series of 55 patients with cholangiocarcinoma, hepatocellular carcinoma, and CRC liver metastases. However, our analysis comparing serum values of CEA and IL-6 showed a relative high *r* value (.544), but the correlation between the two variables was still highly statistically significant ($P < .001$) because of the large number of patients studied. Moreover, in contrast with the study by Goydos et al.,[26] in our subset of patients with stage IV disease (55 patients), serum concentrations of CEA and IL-6 were also significantly correlated. However, because high concentrations of IL-6 have also been described in type of tumors that usually do not produce CEA, such as renal cell carcinoma,[27–29] malignant melanoma,[30] head and neck cancer,[31] epithelial ovarian cancer,[32] and lymphomas,[33,34] other molecules could be involved in the mechanism leading to the induction of IL-6.

In our study, serum concentration of IL-6 was significantly associated with a more advanced tumor stage. Our data confirm those previously reported by Ueda et al.,[35] on a group of 24 patients with CRC showing significantly higher concentrations of IL-6 in patients with liver and lung metastasis, compared with those without distant metastasis. The serum concentration of IL-6 may reflect the total tumor burden. In fact, these data are in accord with the results of studies reporting a correlation between serum concentration of IL-6 and tumor burden in cholangiocarcinoma,[26] esophageal squamous cell carcinoma,[36] gastric carcinoma,[37] and malignant melanoma.[30] It is noteworthy that Piancatelli et al.[38] have recently demonstrated specific IL-6 gene expression at the tumor site in human CRC; however, the expression of IL-6 seems to originate from infiltrating mononuclear cells, because CRC cell lines did not express IL-6 transcripts.

In the present study, a preoperative IL-6 serum concentration of more than 10 pg/ml was a negative prognostic factor of survival, independent from tumor site, grade, and stage. This could be explained because IL-6 may be a potent stimulator of metastasis, up-regulating the expression on endothelial cells of adhesion receptors such as intercellular adhesion molecule-1 and endothelial leukocyte adhesion molecule-1[13–15]; IL-6 also increases the production of hepatocyte growth factor and, indirectly, vascular endothelial growth factor,[16,17] both of which may stimulate tumor progression. Further studies are needed to clarify the source and the mechanism underlying IL-6 production and its effect in patients with cancer. These data, in fact, may be of clinical relevance, because high serum concentrations of IL-6 have been shown to be negative prognostic factors in patients with epithelial ovarian cancer,[32] metastatic renal cell carcinoma,[28,29] melanoma,[30] esophageal squamous cell carcinoma,[36] gastric cancer[37] and lymphomas. [33,34] Our findings also confirm these data in patients with CRC.

In conclusion, in patients with CRC, IL-6 concentrations were associated with high circulating CEA and advanced stage; furthermore, IL-6 blood concentrations of more than 10 pg/ml were independent negative prognostic marker of survival, whereas CEA blood concentrations were not. Based on this evidence, the preoperative dosage of IL-6 could be a useful tool for estimating the risk of recurrence after surgical removal of the primary tumor. This may help to identify early-stage patients more likely to benefit from adjuvant therapy.

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