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Original Article

Study of Interleukin-6 in the Spread of Colorectal Cancer : The Diagnostic Significance of IL-6

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We investigated the diagnostic significance of IL-6 for lymph node metastasis and/or hepatic metastasis from colorectal cancer in 65 patients and evaluated the contributions of 8 factors (IL-6, HGF, IL-1 β, TNF-α, TGF-β1, ELAM-1, ICAM-1, VCAM-1) toward Dukes's classification of 53 patients. We also examined IL-6 expression in tumor tissue. From the receiver operating characteristic (ROC) curve analysis, an optimal cutoff value of 5.8 pg/ml was determined to classify lymph node and/or hepatic metastasis, and that of 6.3 pg/ml was determined to classify hepatic metastasis. These values indicated sensitivities of 55.0% and 71.4%, and specificities of 100% and 88.6%, respectively. IL-6, HGF, and ELAM-1 were very useful for distinguishing among Dukes's A/B group, C group, and D group. In all cases with high IL-6 values (more than 25.0 pg/ml), immunohistochemical staining was positive for IL-6 in the cytoplasm of cancer cells. IL-6 is strongly suspected to be involved in lymph node and/or hepatic metastasis by promoting it through HGF, and serum IL-6 value (pg/ml) would be useful diagnostically to estimate whether or not there is a high risk of lymph node and/or hepatic metastasis.

Key words: IL-6, colorectal cancer, lymph node metastasis, hepatic metastasis, diagnostic significance

A cancer cell has the ability to invade host stroma and to metastasize. Metastasis refers to the ability of cancer cells to invade stroma and to generate secondary tumors at sites distant from the primary tumor. It is well known that the spread of tumors can be enhanced not only by cancer cells but also by interstitial cells. It is becoming clear that various cytokines, which are produced from cancer tissue including interstitial cells, are associated with invasion and metastasis [1, 2]. Cytokines are considered to form a cytokine network, either autocrine or paracrine, and to be involved in the system of invasion and metastasis through receptors expressed on cancer cells. It has been found that interleukin-6 (IL-6) has multiple biological activities [3–6]. We considered the possibility of stimulating cancer cell growth by IL-6, and investigated IL-6's possible role in predicting the spread of tumors, including invasion and metastasis. We have already reported a significant correlation between serum IL-6 values and both lymph node and hepatic metastasis from colorectal cancer, and we have elucidated the role of IL-6 as a prognostic factor [7]. The purpose of the present study was to clarify IL-6's diagnostic significance for

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lymph node metastasis and/or hepatic metastasis and to analyze the serum levels of IL-6, IL-6-related cytokines, and adhesion molecules to distinguish patients with colorectal cancer according to Dukes's classification.

Patients and Methods

Sixty-five patients with colorectal cancer diagnosed histopathologically without pre-treatment, and who underwent surgery at our department between January 1998 and August 2002, were enrolled in this study. The patients consisted of 46 men and 19 women (mean age 65.5 y/o, range 26–89 y/o). Twenty-one patients were considered to have hepatic metastasis clinically (Dukes's D), 8 patients with Dukes's A, 17 with Dukes's B, and 19 with Dukes's C (Table 1).

Variables. In the 65 patients with colorectal cancer, we statistically assessed the preoperative serum values of IL-6 (pg/ml) to be of diagnostic significance for 1) lymph node metastasis and/or hepatic metastasis, and 2) hepatic metastasis. 3) We analyzed the contributions of IL-6, IL-6-related cytokines (hepatocyte growth factor (HGF) (ng/ml), interleukin-1β (IL-1β) (pg/ml), tumor necrosis factor-α (TNF-α) (pg/ml), transforming growth factor-β1 (TGF-β1) (ng/ml)), and adhesion molecules (intercellular adhesion molecule-1 (ICAM-1) (ng/ml), vascular-cellular adhesion molecule-1 (VCAM-1) (ng/ml), and endothelial cell leukocyte adhesion molecule-1 (ELAM-1) (ng/ml)) to apply Dukes's classification to 53 patients. 4) We also examined the expression of IL-6 in tumor tissue in 6 cases with high serum levels (more than 25.0 pg/ml) and in 4 cases with low serum levels (less than 2.0 pg/ml) of IL-6.

Table 1 Patients' characteristics

Variable	Number of patients
Total patients	65
Age (years)	
Median	65.5
Range	26–89
Gender (%)	
Male	46 (70.8)
Female	19 (29.2)
Location (%)	
Cecum	7 (10.8)
Ascending	9 (13.8)
Transverse	8 (12.3)
Descending	1 (1.5)
Sigmoid	14 (21.5)
Rectum	26 (40.0)
Dukes' classification (%)	
A	8 (12.3)
B	17 (26.2)
C	19 (29.2)
n_1 (+)	11 (16.9)
n_2 (+)	5 (7.7)
n_3 (+)	3 (4.6)
D (H' (+))	21 (32.3)

Histological findings were described according to the Japanese Society for Cancer of the Colon and Rectum (7 th edition) [8].

Assay of serum cytokine value. The serum value of IL-6 was measured by chemiluminescent enzyme immunoassay (CLEIA) using a commercial kit (Fujirebio Inc., Tokyo, Japan). Serum values of several cytokines were measured by enzyme-linked immunosorbent assay (ELISA) by commercially available methods: HGF (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), IL-1β (Biosource Europa S.A., Camarillo, CA, USA), TNF-α (Japan Immunoresearch Laboratories Co., Ltd., Gunma, Japan), and TGF-β1 (R&D Systems Inc., Minneapolis, MN, USA).

Immunohistochemical staining. IL-6 was detected by anti-human IL-6 monoclonal antibody (Cosmo Bio Co., Ltd., Tokyo, Japan). Immunohistochemical staining was performed using a labeled streptavidin-biotin (LSAB) kit (Dako Cytomation Tokyo Inc., Tokyo, Japan). IL-6 immunostaining was judged to be positive when more than 10% of cancer cells were detected by immunostaining in the cytoplasm.

Statistical analysis. Receiver operating characteristics (ROC) analysis was used to calculate cutoff values to determine the probability that most adequately classified lymph node and/or hepatic metastasis. The ROC curves were traced using ROCKIT software (Kurt Rossmann Laboratories, Chicago, IL, USA), and the cutoff point, sensitivity, and specificity were measured. We analyzed 8 factors (IL-6, 4 IL-6-related cytokines, and 3 adhesion molecules) to distinguish patients according to Dukes's classification by canonical discriminate analysis, which was conducted in order to distinguish among a

group of samples from potentially different populations. This technique creates canonical varieties from the original variables containing the relevant information for discrimination. Canonical discriminate analysis was calculated using the Statistical Analysis System (SAS) from SAS Institute (Cary, NC, USA).

Results

1. The classification capability of the model from the ROC curve analysis was 0.718 (cutoff point: SE: 0.063, 95% CI: 0.593–0.823), and this cutoff point had a sensitivity of 55.0% (22 of 40 cases), a specificity of 100% (25 of 25 cases), a positive predictive value of 100% (22 of 22 cases), and a negative predictive value of 58.1% (25 of 43 cases) for determining the absence or presence of lymph node metastasis and/or hepatic metastasis. An optimal cutoff value of 5.8 pg/ml was determined to classify them (Fig. 1).

2. The classification capability of the model from the ROC curve analysis was 0.819 (cutoff point: SE: 0.061, 95% CI: 0.704–0.904), and this cutoff point had a sensitivity of 71.4% (15 of 21 cases), a specificity of 88.6% (39 of 44 cases), a positive predictive value of 75.0% (15 of 20 cases), and a negative predictive value of 86.7% (39 of 45 cases) for determining the absence or presence of hepatic metastasis. An optimal cutoff value of 6.3 pg/ml was determined to classify them (Fig. 2).

3. Dukes's D group could be distinguished from the A, B, and C groups by the first canonical axis (a-a'). HGF (0.77) contributed primarily and IL-6 (0.46) contributed secondarily to obtain the first canonical axis. Then, Dukes's C group could be distinguished from the A and B groups by the second canonical axis (b-b'), and the contribution of ELAM-1 (1.01) was strong enough to obtain a second canonical axis. However, these 8 factors did not contribute enough to distinguish between Dukes's A and B groups (Fig. 3).

4. In all 6 cases with high serum levels (more than 25.0 pg/ml) of IL-6, immunohistochemical staining showed positive findings for IL-6 in the cytoplasm of cancer cells. On the other hand, there was no evidence of positive findings for IL-6 in the 4 cases with low serum levels (less than 2.0 pg/ml) of IL-6.

Discussion

In 1986, the nucleotide deduced amino acid sequences of cloned human IL-6 cDNA were purified. Human IL-6 consists of 212 amino acids, including a

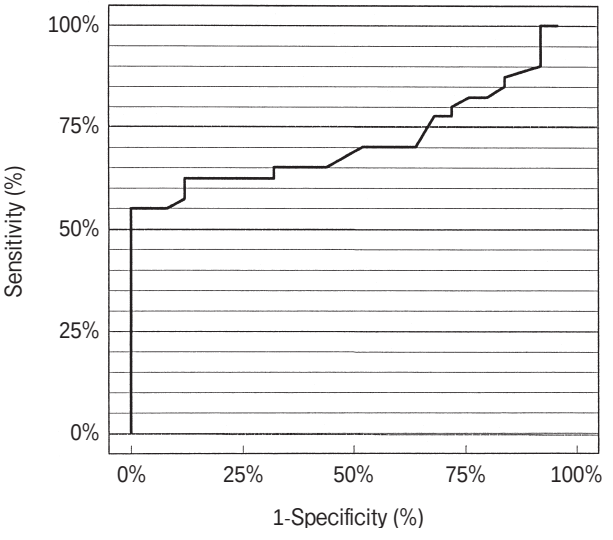

Fig. 1 ROC curve traced using ROCKIT software for lymph node and/or hepatic metastasis by the preoperative serum IL-6 values.

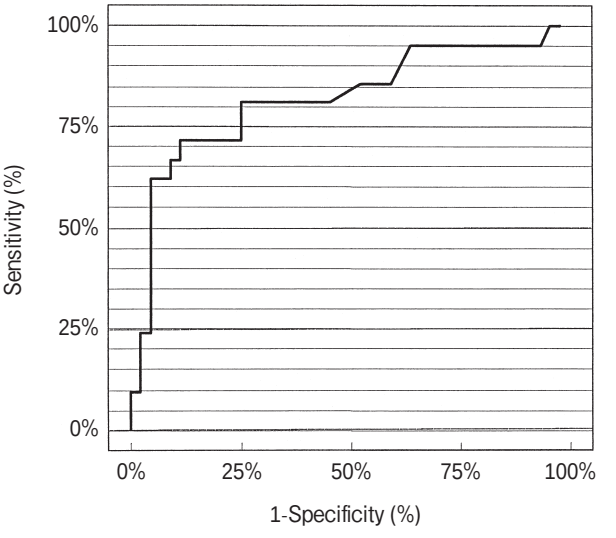

Fig. 2 ROC curve traced using ROCKIT software for hepatic metastasis by the preoperative serum IL-6 values.

hydrophobic signal sequence of 28 amino acids [9–11]. On the other hand, the receptor system of IL-6 consists of an 80 kD glucoprotein (IL-6R) and a 130 kD glucoprotein (gp 130), and signals are transferred into cells by IL-6 combined with IL-6R on the cell membrane [12]. We considered the possibility of stimulating cancer cell growth by IL-6, and investigated the possible role of IL-6 in predicting the spread of tumors, including invasion and metastasis. Actually, we have already reported a significant relationship ($r = 0.43074$, $p < 0.01$) between serum IL-6 values and Dukes's classification, and have also showed significant correlations between IL-6 and both lymph node metastasis ($r = 0.46864$, $p < 0.01$) and hepatic metastasis (Fisher prob = 0.000001, $p < 0.01$) [7].

Cancer metastasis is a complex process involving coordinated cellular responses of both cancer cells and normal cells. Metastasis involves several steps: 1) invasion of the stroma, 2) intravasation of the blood vessel, 3) circulation in the blood, 4) lodging and adhesion in target capillaries, 5) extravasation from the blood vessels, and 6) proliferation of secondary tumors [13]. IL-6 is thought to be involved in steps 1, 2, and 3.

As proof of the mechanism of IL-6 involved in steps 1, 2, and 3, Tamm *et al.* showed that IL-6 increases the motogenic activity of cancer cells by the autocrine pathway (that is, IL-6 secreted from the cancer cells combines with IL-6R, which is expressed on the surface of cancer cells; together, IL-6 and IL-6R act on cancer cells directly.) [14]. Subsequent research showed clearly that a paracrine pathway is involved in the effects of HGF and IL-6 on the invasion and metastasis of cancer cells [15–17]. When IL-6 produced by cancer cells stimulates interstitial cells to secrete HGF, HGF combines with the HGF receptor (c-Met) expressed on cancer cells. HGF raises the motogenic activity of these cells, and it is thought that the cancer cells move to the metastasis site [18, 19]. Actually, there was a significant relationship ($r = 0.53226$, $p < 0.01$) between IL-6 and HGF in our research [7], and IL-6 may act through HGF on cancer cells by the promotion and acceleration of developing invasion and lymph node metastasis and/or hepatic metastasis.

It has been reported that ICAM-1, VCAM-1, and ELAM-1 are expressed on endothelial cells by proinflammatory cytokines such as IL-1β and TNF-α, which are located upstream of IL-6 [20, 21] and which, combined with IL-6, promote the adhesion of cancer cells and endothelial cells [22]. However,

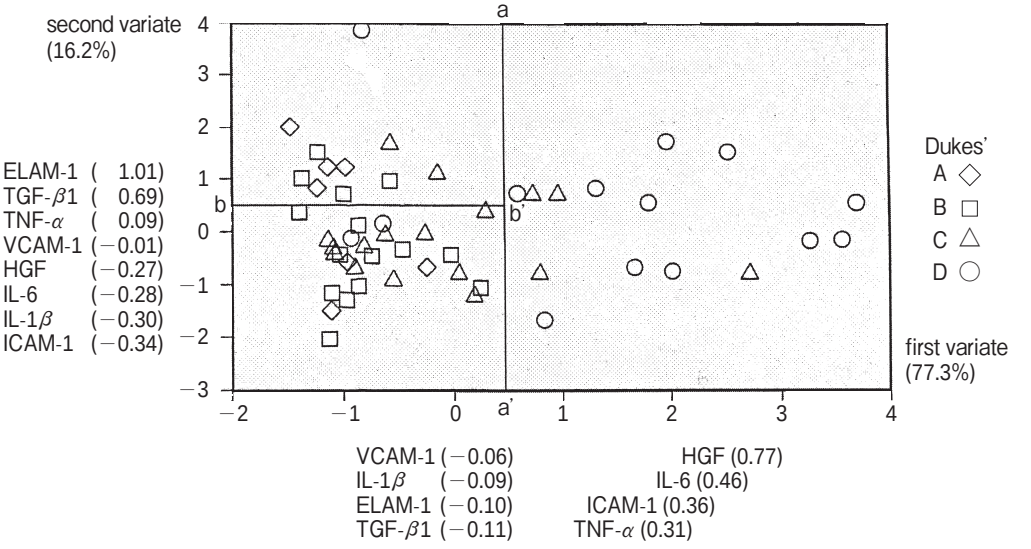

Fig. 3 Fifty-three patients are distinguished according to Dukes's classification by canonical discriminant analysis. Dukes's D group can be distinguished from Dukes's A, B, and C groups by the first canonical axis (a-a'). Dukes's C group can be distinguished from Dukes's A and B groups by the second canonical axis (b-b').

IL-6 did not correlate significantly with IL-1β ($r = 0.0517$, NS) or TNF-α ($r = 0.12136$, NS) in our research [7].

Although specific IL-6 immunostaining was observed in the cytoplasm of cancer cells in all 6 patients with high IL-6 values (more than 25.0 pg/ml), IL-6 immunostaining was not shown in 4 patients with low IL-6 values (less than 2.0 pg/ml). This strongly suggested that the high serum value of IL-6 reflects IL-6 secretion by cancer cells. However, it remains unclear what up-regulates IL-6 production in cancer cells. Experimental studies have recently demonstrated that *in vitro* treatment of Kupffer cells with carcinoembryonic antigen (CEA) induces expression of cytokines such as IL-1α, IL-1β, IL-6, and TNF-α [22]. In addition, the injection of CEA into mice results in a significant dose-dependent IL-6 response [23]. Furthermore, Belluco *et al.* reported that the preoperative serum concentration of IL-6 was associated with CEA in 208 patients with colorectal cancer [24]. These data suggest that CEA induces the systemic production of IL-6. In fact, a significant relationship between IL-6 and CEA was found in the present study (data not shown; Spearman rank correlation = 0.59726, $p < 0.01$).

Because IL-6 is affected by various clinical conditions, such as sepsis, trauma, surgery, inflammation, and other forms of chronic stress, its serum level may reflect a lot of indefinite bias. It is therefore not necessarily suitable as a screening modality to rule out malignancy. Our results suggest that the clinical significance of IL-6 is limited to evaluating the spread of colorectal cancer, and that IL-6 should be used as a tumor marker for lymph node metastasis and/or hepatic metastasis. In general, ROC analysis can be employed to assess performance in any 2-group classification task. The ROC curve represents the relationship between 'true positive fraction' (TPF) and the 'false positive fraction' (FPF). In the terminology of medical diagnosis, TPF is equivalent to 'sensitivity', whereas FPF is equivalent to 1-'specificity'. The ROC curve displays the trade-off between sensitivity and specificity that a diagnostic test allows as the cutoff between nominally negative and nominally positive test results (that is, the threshold of abnormality) varies [25, 26]. Actually, when the optimal cutoff values of IL-6 were set at 5.8 pg/ml with regard to the diagnostic significance of serum IL-6 values for lymph node metastasis and/or hepatic metastasis, and to 6.3 pg/ml for hepatic metastasis, the sensitivity values were 55.0% and 71.4% and the specificity values were 100% and 88.6%, respectively. Although it cannot be said that the sensitivity is high enough, the specificity is high and is not inferior to those for other tumor markers, such as CEA and carbohydrate antigen (CA) 19–9 [27]. Therefore, serum IL-6 may be valuable as a prognostic factor for survival, and it is recommended for the preoperative prediction of disease stage. However, the influence of IL-6, as well as the influences of related factors, were not clear enough to allow us to evaluate tumor invasion from the results of canonical discriminate analysis. In patients with colorectal cancer who showed high levels of C-reactive protein (CRP) before surgery (in this research, 17 examples (81.0%) showed a rise of CRP among 21 IL-6 high-value patients) [28, 29], examination of serum IL-6 level and IL-6 expression in cancer cells would be diagnostically useful for estimating whether or not there is a high risk of lymph node metastasis and/or hepatic metastasis.

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