Surg Today (2017) 47:483–489
DOI 10.1007/s00595-016-1404-7

ORIGINAL ARTICLE

High serum levels of interleukin-6 in patients with advanced or metastatic colorectal cancer: the effect on the outcome and the response to chemotherapy plus bevacizumab

Masayasu Hara[1] · Takaya Nagasaki[1] · Kazuyoshi Shiga[1] · Hiroki Takahashi[1] · Hiromitsu Takeyama[1]

Received: 23 January 2016 / Accepted: 12 July 2016 / Published online: 22 August 2016

Abstract
Purpose We evaluated the relationship of the pretreatment serum IL-6 levels with the outcome and treatment response in patients with advanced or metastatic colorectal cancer (CRC) who underwent bevacizumab-containing chemotherapy.
Methods In this retrospective study, the pretreatment serum IL-6 and plasma vascular endothelial growth factor (VEGF) levels were measured in 113 patients with metastatic CRC. The cut-off values for these measurements, as determined by a receiver operating characteristic curve analysis, were 4.3 and 66 pg/mL, respectively. The median follow-up period was 19 months (range 1–40 months). Sixty-three patients had primary cancer, and 38 had a metachronous recurrence. Thirty patients underwent curative resection, and 71 underwent chemotherapy, 53 of whom received bevacizumab-containing chemotherapy. Overall survival (OS) and progression-free survival (PFS) were estimated using Kaplan–Meier and multivariate Cox proportional hazards regression analyses.
Results The plasma VEGF levels and positive *KRAS* mutation status were not associated with the outcomes. However, high serum IL-6 levels were significantly associated with poorer OS and PFS in comparison to low serum IL-6 levels. A Cox proportional hazards regression analysis showed that high serum IL-6 levels were an independent risk factor for a poor outcome.
Conclusion In patients with metastatic CRC, high pretreatment serum IL-6 levels were associated with a poor outcome and bevacizumab resistance.

Keywords Colorectal cancer · Bevacizumab · Interleukin-6 (IL-6) · Chemo-resistance

Introduction

Angiogenesis is an essential survival mechanism for many tumorous cancers, and antiangiogenic drugs are included in various chemotherapy regimens. Because vascular endothelial growth factor (VEGF) is a potent angiogenic factor, bevacizumab, an anti-VEGF antibody, is a clinically beneficial treatment for patients with colorectal, lung, and renal cancer [1, 2].

Bevacizumab is widely used, and although its mechanisms of action against cancer have been well established, the mechanisms underlying resistance, such as that seen in gastric cancers [3], remain unknown. Furthermore, continued therapy can lead to acquired resistance, even in patients with bevacizumab-sensitive cancer [4, 5]. At present, there are no effective biomarkers to determine the tumor response to bevacizumab. Although the plasma VEGF level and *KRAS* mutations, which themselves exert an upstream effect on VEGF secretion [6], have been implicated, their effects on the tumor response to bevacizumab are debatable.

Interleukin-6 (IL-6), which promotes the secretion of hepatic C-reactive protein, is an inflammatory cytokine secreted by immune cells and adipose tissue. Previous studies have reported that some inflammatory cytokines, including IL-6, have an angiogenic effect [7]. Furthermore, inflammation has been correlated with the prognosis, and high

✉ Masayasu Hara
mshara@med.nagoya-cu.ac.jp

[1] Department of Gastroenterological Surgery, Nagoya City University, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan

pretreatment serum IL-6 levels have been shown to influence the response to neoadjuvant bevacizumab-containing chemotherapy [8]. Moreover, combination therapy with anti-VEGF and anti-IL-6 therapies has a potent inhibiting effect on tumor growth [7, 9, 10]. We previously demonstrated that the presence of serum IL-6 influences the recurrence of CRC [11]. Accordingly, this study aimed to investigate the relationship between IL-6 and the prognosis of patients with advanced or metastatic CRC by examining the effect of the pretreatment serum IL-6 levels on the outcome and the treatment response in patients undergoing anti-VEGF therapy with bevacizumab.

Patients and methods

One-hundred one CRC patients with either distant metastasis or unresectable lesions who were treated with anticancer therapies were enrolled in this retrospective study. The inclusion criterion was the presence of either a primary cancer with distant metastasis or metachronous metastasis at the time of their diagnosis. The median patient age was 67.0 ± 9.1 years (range 45–87 years), and the male-to-female ratio was 54:47. Sixty-three patients had primary CRC, and 38 had metachronous recurrent cancer. Thirty patients underwent curative resection and 71 underwent non-curative resection with subsequent chemotherapy. Of these 71 patients, 60 were treated with oxaliplatin-based chemotherapy (FOLFOX, 42; XELOX, 18), and 11 received a FOLFIRI regimen. In addition, of these 71 patients, 53 received bevacizumab-containing chemotherapy as the first-line treatment (Tables 1, 2).

The *KRAS* mutation status was evaluated in patients who underwent chemotherapy. Mutations were noted in 26 (36.6 %) patients (codon 12, 20 patients; codon 13, 6 patients). The pretreatment serum carcinoembryonic antigen (CEA), serum IL-6, and plasma VEGF levels were measured in all 101 patients. The median follow-up was 19 months (range 1–40 months). The patients were evaluated every 3–6 months using computed tomography (CT).

Statistical analyses

The prospectively collected data were analyzed retrospectively. A univariate analysis of the IL-6 and VEGF levels and the KRAS mutation status was performed to determine the effect of these factors on overall survival (OS). The cut-off values for IL-6 and VEGF were determined in 185 stage I–III patients who underwent curative surgery in our hospital between 2010 and 2013. We assumed death as the endpoint, and determined the optimal cut-off values for serum IL-6 and plasma VEGF as 4.3 and 66 pg/mL, respectively (Fig. 1). Based on these cut-off values, 51 and 50 patients were classified into the low and high IL-6 groups, respectively, and 62 and 39 patients were classified into the low and high VEGF groups, respectively. The correlations between the IL-6 and VEGF levels were estimated using the Pearson correlation coefficient.

Table 1 The clinicopathological features of patients with colorectal cancer

	Total	Percentage
Age (years)[a]	67.0 ± 9.1	
Sex		
Male	54	53.5
Female	47	46.5
Serum IL-6 (pg/mL)[a]	4.3 ± 300	
Plasma VEGF (pg/mL)[a]	54.5 ± 162	
KRAS[b]		
Wild type	45	63.4
Mutation	26	36.6
Serum CEA (ng/mL)[a]	7.6 ± 129.8	
Follow-up period (months)	19.0 ± 11.2	
Presence of primary lesion		
Yes	63	62.4
No	38	37.6

IL-6 interleukin 6, *VEGF* vascular endothelial growth factor, *CEA* carcinoembryonic antigen

[a] Median ± SD

[b] Evaluated in 71 patients who underwent chemotherapy

To evaluate the treatment response, the rate of progression-free survival (PFS) was determined in the 71 patients who underwent chemotherapy for unresectable cancer. Tumor progression was classified according to Response Evaluation Criteria in Solid Tumors version 1.1. The patients' lesions were assessed every 3 months using CT. To determine the predictors of the outcome and treatment response, a multivariate analysis was performed for the 71 patients who underwent chemotherapy. The carcinoembryonic antigen level, sex, age, the presence of a primary tumor, the IL-6 and VEGF levels, and the *KRAS* mutation status were evaluated.

Identical analyses were performed in the 53 patients who were treated with bevacizumab-containing chemotherapy.

Survival was estimated using Kaplan–Meier and multivariate Cox proportional hazards regression analyses. p values of <0.05 were considered to indicate statistical significance.

Results

The measured mediator values

In the 101 patients with available data, the mean serum IL-6 and plasma VEGF levels were 13.7 and 104.2 pg/mL,

Table 2 The characteristics of the patients who underwent chemotherapy

	Chemotherapy ($n = 71$)	Bevacizumab-containing chemotherapy ($n = 53$)	%
Age (years)[a]	67 ± 8.9	65.0 ± 7.3	
Sex (M:F)	36:35	22:31	
Serum CEA (ng/mL)[a]	8.1 ± 1125.3	13.1 ± 1272.3	
Serum IL-6 (pg/mL)[a]	5.2 ± 35.1	4.5 ± 18.2	
Plasma VEGF (pg/mL)[a]	59.0 ± 189.3	65.0 ± 146.0	
KRAS (W:M)	45:26	31:22	
Chemotherapy			
mFOLFOX6	42	28	59.2
XELOX	18	16	25.4
FOLFIRI	11	9	15.5

IL-6 interleukin 6, *VEGF* vascular endothelial growth factor, *CEA* carcinoembryonic antigen, *W* wild type, *M* mutant

[a] Median ± SD

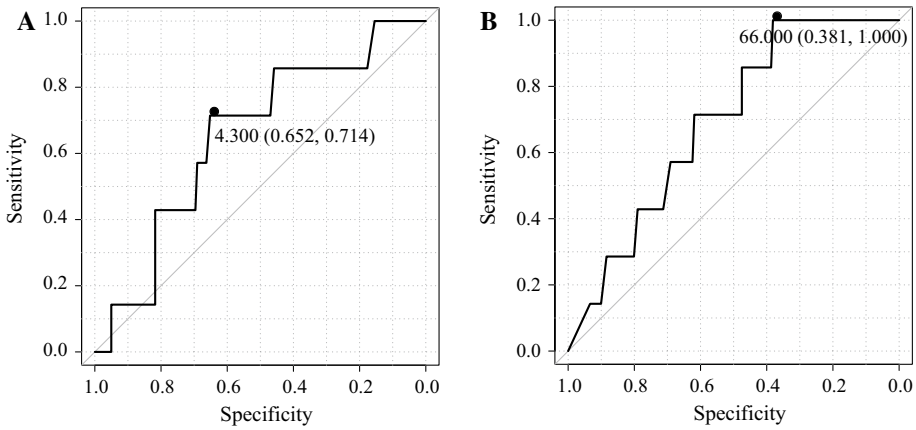

Fig. 1 The receiver operating characteristics curve for IL-6 (**a**) and VEGF (**b**) according to the survival of 185 stage I–III patients. The optimal cut-off values for IL-6 and VEGF were 4.3 and 66 pg/mL, respectively

respectively. There was no correlation between the IL-6 and VEGF levels ($r = 0.16$; $p = 0.12$; Fig. 2).

Overall survival

Twenty-seven patients (26.7 %) died of cancer. Among the whole population of 101 patients, those with high serum IL-6 levels had significantly worse 3-year OS rates ($n = 51$; 21.3 %) than those with low serum IL-6 levels ($n = 50$; 71.4 %; $p = 0.02$; Fig. 3a). The plasma VEGF level (high VEGF, $n = 39$; 51.4 %; low VEGF, $n = 62$; 49.7 %; $p = 0.8$) and KRAS mutation status (KRAS wild type, $n = 45$; 34 %; KRAS mutant, $n = 26$; 23.3 %; $p = 0.7$) had no significant effect on the 3-year OS rates.

Progression-free survival

The PFS curves of the 71 patients who underwent chemotherapy, stratified according to the pretreatment IL-6 and VEGF levels and the *KRAS* mutation status, are shown in Fig. 4a–c. The plasma VEGF level and KRAS mutation

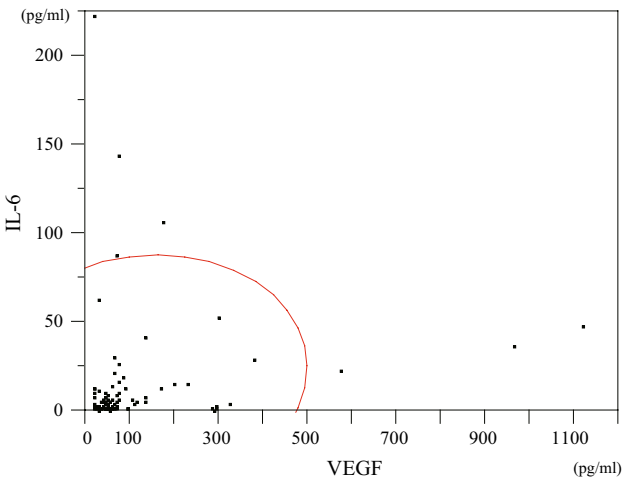

Fig. 2 The measured serum IL-6 and plasma VEGF values. The Pearson correlation coefficient was 0.16, and the p value was 0.12

status did not affect PFS (high VEGF, $n = 31$, 19.1 months; low VEGF, $n = 40$, 17.2 months; KRAS wild type, $n = 45$, 19.1 months; KRAS mutant, $n = 26$, 10.6 months). In

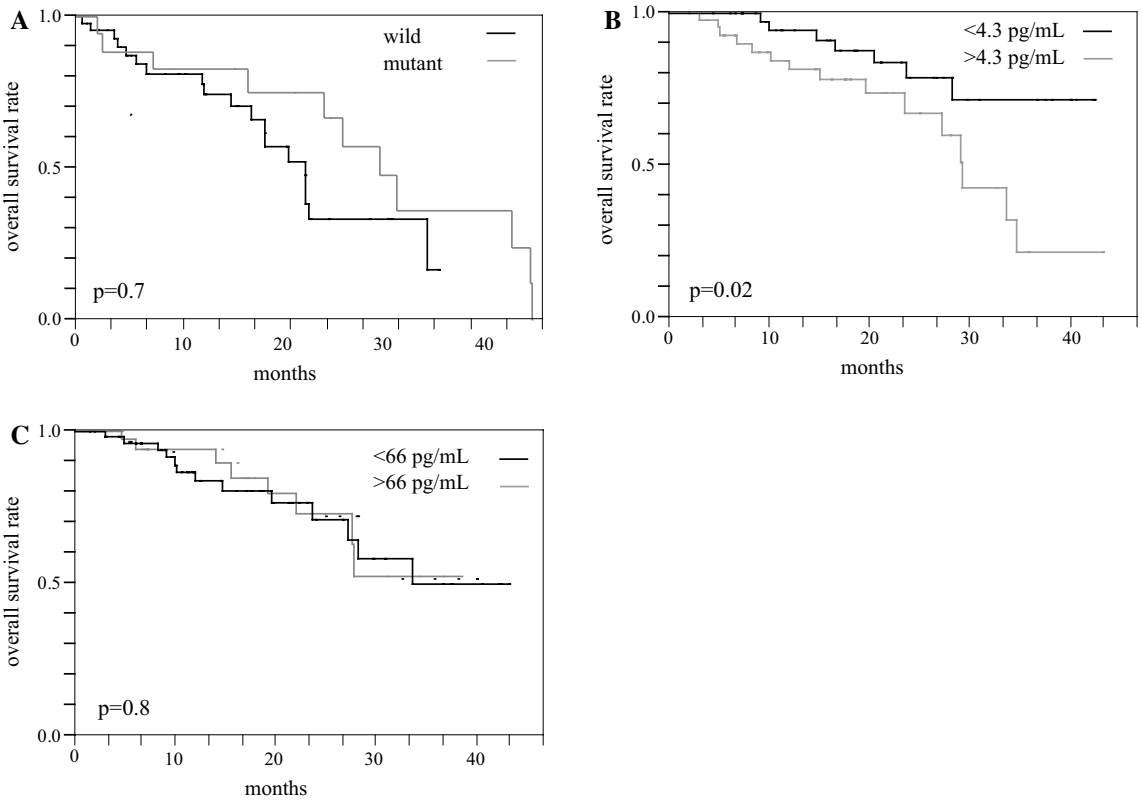

Fig. 3 The overall survival of 101 patients with colorectal cancer stratified according to the *KRAS* mutation status (**a**), the serum IL-6 level (**b**), and the plasma VEGF level (**c**). Although neither the KRAS mutation status nor the plasma VEGF level had an effect on overall survival, the serum IL-6 had a significant effect on overall survival. Patients with high serum IL-6 levels had significantly worse outcomes in comparison to those with low serum IL-6 levels

contrast, the patients with high serum IL-6 levels had a significantly poorer median PFS [$n = 31$, 10.0 months; 95 % confidence interval (CI) 6.5–19.2 months] in comparison to those with low serum IL-6 levels ($n = 40$, 24.7 months; 95 % CI 10.6–26.9 months; $p = 0.006$).

Among the 53 patients who received bevacizumab-containing chemotherapy as the first-line treatment, those with high serum IL-6 levels showed a poorer median PFS ($n = 27$, 9.8 months) than those with low serum IL-6 levels ($n = 26$, median PFS, 24.7 months; Fig. 4d–f). In contrast, the plasma VEGF level and KRAS mutation status had no significant effect on the median PFS (high VEGF, $n = 24$, 13.6 months; low VEGF, $n = 29$, 11 months; KRAS wild type, $n = 31$, 10.0 months; KRAS mutant, $n = 22$, 13.6 months; both $p = 0.3$).

Response to bevacizumab treatment

The PFS of the 71 patients who received chemotherapy for unresectable lesions was analyzed as a measure of the treatment response using a Cox proportional hazards model. A high serum IL-6 level was a prognostic factor for PFS ($p = 0.0001$), whereas the *KRAS* mutation status and plasma VEGF level were not significant prognostic factors for PFS ($p = 0.2$ and 0.4, respectively; Table 3).

The PFS rate of 53 patients who received a first-line bevacizumab-containing chemotherapy was also evaluated using a multivariate analysis in a Cox proportional hazards model. A high serum IL-6 level was a prognostic factor for PFS ($p = 0.002$), whereas the *KRAS* mutation status and plasma VEGF level were not significant prognostic factors for PFS ($p = 0.7$ and 0.08, respectively; Table 4).

Discussion

Antiangiogenic therapy is a mainstay treatment for patients with CRC [12]. However, despite the early initial responses to bevacizumab in individual patients, the overall clinical benefits, as reflected by PFS and OS, have been modest. Thus, there is a growing interest in understanding the mechanisms of acquired resistance against the antiangiogenic effects of bevacizumab. One mechanism is thought to be the existence of multiple alternative proangiogenic pathways (i.e., platelet-derived growth factors, phosphatidylinositol-glycan biosynthesis class F proteins, and fibroblast

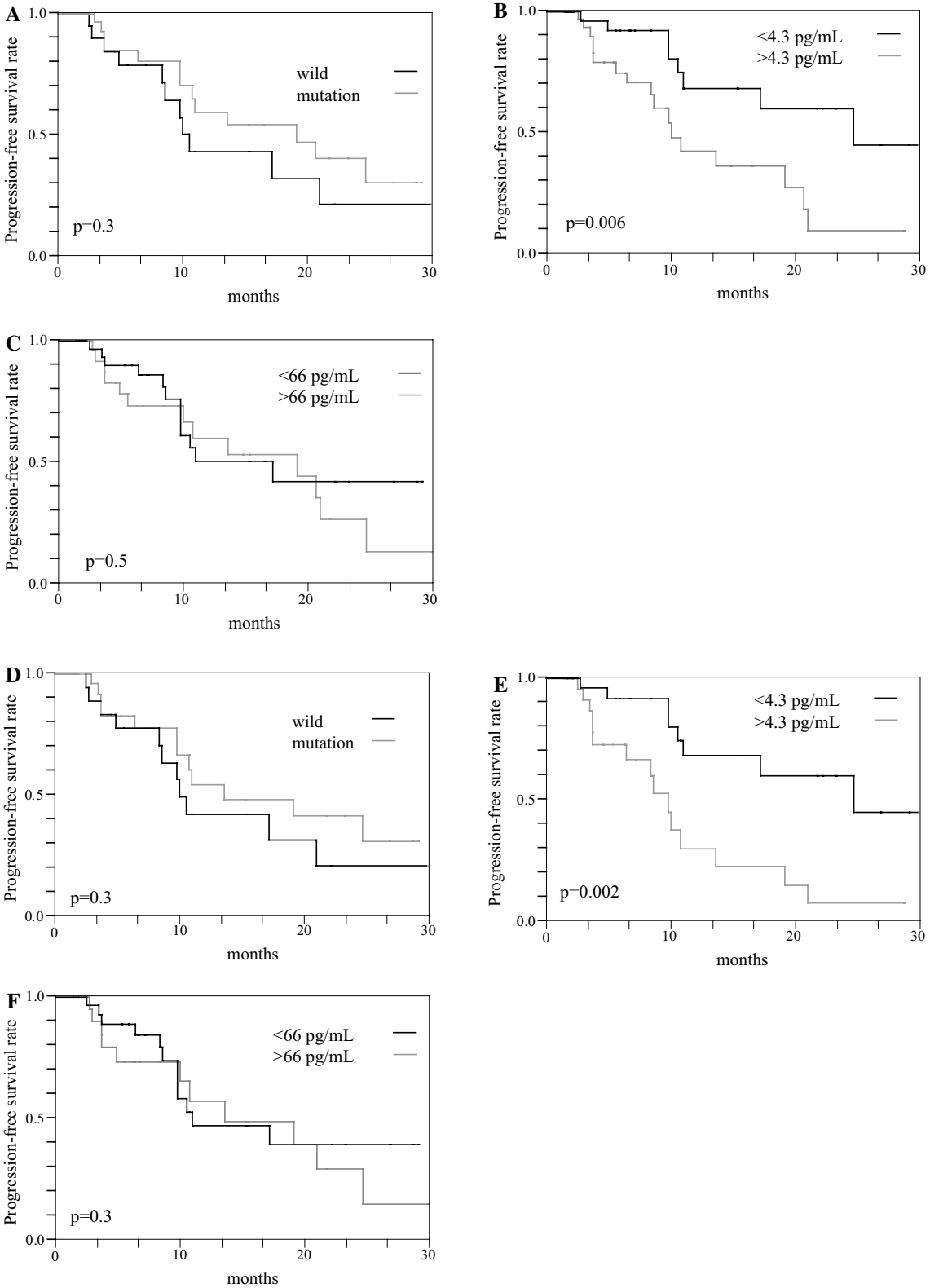

Fig. 4 The progression-free survival (PFS) of 71 patients with colorectal cancer who underwent chemotherapy stratified according to the *KRAS* mutation status (**a**), serum IL-6 level (**b**), and plasma VEGF level (**c**). The PFS of 53 patients who underwent bevacizumab-containing chemotherapy stratified according to *KRAS* mutation status (**d**), serum IL-6 level (**e**), and plasma VEGF level (**f**). Patients with high serum IL-6 levels had worse outcomes than those with low serum IL-6 levels. Neither the *KRAS* mutation status nor the plasma VEGF level had an effect on progression-free survival

Table 3 The multivariate analysis (Cox proportional hazards model) for the 71 patients who underwent chemotherapy

	Risk ratio	95 % CI	*p* value
Age (>65 years old)	0.7	0.4–1.2	0.2
Male	1.0	0.6–1.7	0.7
Primary tumor	0.6	0.4–1.2	0.1
High serum IL-6 (>4.3 pg/mL)	2.6	1.5–4.8	0.0001
High plasma VEGF (>66 pg/mL)	0.8	0.6–4.8	0.4
KRAS mutation	1.4	0.9–2.5	0.2
CEA (>5 ng/mL)	0.9	0.6–1.7	0.9

CI confidence interval, *IL-6* interleukin 6, *VEGF* vascular endothelial growth factor, *CEA* carcinoembryonic antigen

Table 4 The multivariate analysis (Cox proportional hazards model) for the 53 patients who underwent bevacizumab-containing chemotherapy

	Risk ratio	95 % CI	*p* value
Age (>65 years old)	0.8	0.4–1.3	0.3
Male	1.1	0.7–1.7	0.7
Primary tumor	0.7	0.4–1.2	0.1
High serum IL-6 (>4.3 pg/mL)	2.1	1.3–3.6	0.002
High plasma VEGF (>66 pg/mL)	0.9	0.6–1.4	0.7
KRAS mutation	1.5	0.9–2.6	0.08
CEA (>5 ng/mL)	1.2	0.6–2.1	0.6

CI confidence interval, *CEA* carcinoembryonic antigen, *IL-6* interleukin 6, *VEGF* vascular endothelial growth factor

growth factors), which can compensate for the loss of VEGF signaling. In addition, tumors may acquire resistance because of the induction of tumor hypoxia by VEGF signaling inhibition, which includes the upregulation of alternative proangiogenic mediators, such as basic fibroblast growth factor and stromal cell-derived factor α, which might allow for persistent neovascularization, despite continuous bevacizumab therapy.

Previous studies have attempted to demonstrate the predictive value of serum VEGF. Some studies have suggested a correlation between the plasma VEGF levels and the prognosis [13, 14], whereas others have found no correlations. Although bevacizumab is an anti-VEGF antibody, many studies in patients with CRC have reported that the plasma VEGF level did not affect the efficacy of bevacizumab. This study in patients with colon cancer demonstrated that the serum IL-6 level but not the plasma VEGF level affected the tumoral response to bevacizumab [15]. Similar to the previous reports, this study found that the plasma VEGF level had no significant effect on the OS or PFS of CRC patients who received bevacizumab treatment. KRAS mutations have been reported as indicators of the efficacy of bevacizumab [16], because the extracellular signal-regulated kinase levels downstream of KRAS were correlated with the secretion of VEGF.

Willet et al. demonstrated that high serum IL-6 levels were associated with a poor prognosis in patients who received neoadjuvant chemoradiation with bevacizumab [8]. In addition, a high serum IL-6 level has been reported to be a biomarker of a poor response to sunitinib [17, 18].

To date, a few studies have reported that IL-6, or the signal transducer and activator of transcription 3, a downstream mediator of the IL-6 activation pathway, are associated with VEGF secretion [19]. We previously demonstrated that in the presence of cancer cells or inflammatory factors, such as limulus anti-lipopolysaccharide or tumor necrosis factor, cancer-associated fibroblasts (CAF) secrete VEGF [20–22]. Furthermore, we clarified that tumor progression and angiogenesis were suppressed by tocilizumab, an anti-IL-6 receptor antibody, which has been approved for use in the treatment of rheumatoid arthritis [23]. The present data suggest two hypotheses. First, the IL-6-mediated secretion of VEGF by CAFs might be associated with angiogenesis. However, if IL-6-mediated VEGF secretion was the cause of bevacizumab resistance, then a high plasma VEGF level would also be expected to affect the response to bevacizumab. Second, other IL-6-derived angiogenic factors might affect the efficacy of bevacizumab. Further studies are, therefore, needed to clarify the mechanism of IL-6-induced bevacizumab resistance. Both hypotheses suggest a possible clinical indication for the use of tocilizumab in the treatment of CRC, and some experimental reports have suggested an anticancer effect of the drug [9, 10, 24, 25]; however, there are currently no clinical data to support the preclinical data.

This study is associated with some limitations, including the small study population; however, we believe that this study demonstrated meaningful results. Further studies in a larger population of CRC patients and with the inclusion of additional cytokine mediators will be needed to confirm the effects of IL-6 on the outcome and response to bevacizumab treatment.

Conclusions

This study demonstrated that CRC patients with who had high serum IL-6 levels had poorer OS and PFS in comparison to patients with low serum IL-6 levels. The addition of bevacizumab to chemotherapy did not provide a benefit to patients with high serum IL-6 levels, which suggests that these patients may be resistant to anti-VEGF therapy.

Compliance with ethical standards

Conflict of interest None.

References

1. Hainsworth JD, Spigel DR, Burris HA 3rd, Waterhouse D, Clark BL. Whorf R Phase II trial of bevacizumab and everolimus in patients with advanced renal cell carcinoma. J Clin Oncol. 2010;28:2131–6.
2. Wang Y, Deng G, Liu X. Cho WC Monoclonal antibodies in lung cancer. Expert Opin Biol Ther. 2013;13:209–26.
3. Ohtsu A, Shah MA, Van Cutsem E, Rha SY, Sawaki A, Park SR, et al. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. J Clin Oncol. 2011;29:3968–76.
4. Selvakumaran M, Yao KS, Feldman MD. O'Dwyer PJ Antitumor effect of the angiogenesis inhibitor bevacizumab is dependent on susceptibility of tumors to hypoxia-induced apoptosis. Biochem Pharmacol. 2008;75:627–38.
5. Li JL, Sainson RC, Shi W, Leek R, Harrington LS, Preusser M, et al. Delta-like 4 Notch ligand regulates tumor angiogenesis, improves tumor vascular function, and promotes tumor growth in vivo. Cancer Res. 2007;67:11244–53.
6. Tural D, Ozturk M, Selcukbiricik F, Yildiz O, Elicin O, Turna H, et al. Preoperative chemoradiotherapy improves local recurrence free survival in locally advanced rectal cancer. J BUON. 2013;18:385–90.
7. Middleton K, Jones J, Lwin Z. Coward JI Interleukin-6: an angiogenic target in solid tumours. Crit Rev Oncol Hematol. 2014;89:129–39.
8. Willett CG, Duda DG, di Tomaso E, Boucher Y, Ancukiewicz M, Sahani DV, et al. Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: a multidisciplinary phase II study. J Clin Oncol. 2009;27:3020–6.
9. Saidi A, Hagedorn M, Allain N, Verpelli C, Sala C, Bello L, et al. Combined targeting of interleukin-6 and vascular endothelial growth factor potently inhibits glioma growth and invasiveness. Int J Cancer. 2009;125:1054–64.
10. Chalam KV, Grover S, Sambhav K, Balaiya S, Murthy RK. Aqueous interleukin-6 levels are superior to vascular endothelial growth factor in predicting therapeutic response to bevacizumab in age-related macular degeneration. J Ophthalmol. 2014;2014:502174.
11. Shiga K, Hara M, Nagasaki T, Sato T, Takahashi H, Sato M, et al. Preoperative serum interleukin-6 is a potential prognostic factor for colorectal cancer, including stage II patients. Gastroenterol Res Pract. 2016;2016:9701574.
12. Sakurai T, Kudo M. Signaling pathways governing tumor angiogenesis. Oncology. 2011;81(Suppl 1):24–9.
13. Jurgensmeier JM, Schmoll HJ, Robertson JD, Brooks L, Taboada M, Morgan SR, et al. Prognostic and predictive value of VEGF, sVEGFR-2 and CEA in mCRC studies comparing cediranib, bevacizumab and chemotherapy. Br J Cancer. 2013;108:1316–23.
14. Bruhn MA, Townsend AR, Khoon Lee C, Shivasami A, Price TJ, Wrin J, et al. Proangiogenic tumor proteins as potential predictive or prognostic biomarkers for bevacizumab therapy in metastatic colorectal cancer. Int J Cancer. 2014;135:731–41.
15. Hegde PS, Jubb AM, Chen D, Li NF, Meng YG, Bernaards C, et al. Predictive impact of circulating vascular endothelial growth factor in four phase III trials evaluating bevacizumab. Clin Cancer Res. 2013;19:929–37.
16. Asghar U, Hawkes E, Cunningham D. Predictive and prognostic biomarkers for targeted therapy in metastatic colorectal cancer. Clin Colorectal Cancer. 2010;9:274–81.
17. Porta C, Paglino C, Imarisio I, Ganini C, Sacchi L, Quaglini S, et al. Changes in circulating pro-angiogenic cytokines, other than VEGF, before progression to sunitinib therapy in advanced renal cell carcinoma patients. Oncology. 2013;84:115–22.
18. Riehle KJ, Campbell JS, McMahan RS, Johnson MM, Beyer RP, Bammler TK, et al. Regulation of liver regeneration and hepatocarcinogenesis by suppressor of cytokine signaling 3. J Exp Med. 2008;205:91–103.
19. Niu G, Wright KL, Huang M, Song L, Haura E, Turkson J, et al. Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. Oncogene. 2002;21:2000–8.
20. Nagasaki T, Hara M, Nakanishi H, Takahashi H, Sato M. Takeyama H Interleukin-6 released by colon cancer-associated fibroblasts is critical for tumour angiogenesis: anti-interleukin-6 receptor antibody suppressed angiogenesis and inhibited tumour-stroma interaction. Br J Cancer. 2014;110:469–78.
21. Shiga K, Hara M, Nagasaki T, Sato T, Takahashi H, Takeyama H. Cancer-associated fibroblasts: their characteristics and their roles in tumor growth. Cancers. 2015;7:2443–58.
22. Nagasaki T, Hara M, Shiga K, Takeyama H. Relationship between inflammation and cancer progression: recent advances in interleukin-6 signaling and its blockage in cancer therapy. Recept Clin Investig. 2014. doi:10.14800/rci.14202.
23. Forger F, Villiger PM. Treatment of rheumatoid arthritis during pregnancy: present and future. Expert Rev Clin Immunol. 2016;27:1–8.
24. Chen X, Wang L, Wang W, Zhao L, Shan B. B7–H4 facilitates proliferation of esophageal squamous cell carcinoma cells through promoting IL-6/STAT3 pathway activation. Cancer Sci. 2016;107:944–54.
25. Stanam A, Love-Homan L, Joseph TS, Espinosa-Cotton M, Simons AL. Upregulated interleukin-6 expression contributes to erlotinib resistance in head and neck squamous cell carcinoma. Mol Oncol. 2015;9:1371–83.