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Enhancement of the Marginal Area in Colorectal Cancer Liver Metastasis on Computed Tomography Correlates With Microvessel Density and Clinicopathological Factors

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Abstract. *Background: Previously, we aimed to predict the effect of bevacizumab in liver metastasis using the ratio of the computed tomography (CT) value for hepatic metastatic lesions in the arterial phase in contrast-enhanced (CE) CT to that on plain CT. However, there is no report on the relation between the CT contrast effect and microvessel density (MVD) in liver metastasis. Patients and Methods: Thirty-two patients who underwent liver resection for metastasis from colorectal cancer (excluding neoadjuvant chemotherapy cases) between April 2006 and October 2011 at our Department were analyzed retrospectively. The relation between the CE ratios obtained from the whole tumor or tumor margin and the MVD using the liver metastatic lesion of colorectal cancer were analyzed. It was also examined whether the CE ratios and MVD were related to the clinicopathological factors of the primary tumor. Results: There was a significant correlation between the CE ratios obtained from assessment using the entire tumor and the tumor margin as regions of interest (ROI). Furthermore, there was a significant correlation between the MVD and CE ratios. Cases with lymphatic invasion, N2, N3 nodal status and grade B and C were significantly more often observed in the group with high MVD. In contrast, in the group with high CE using the whole tumor as ROI, cases with grade B or C were significantly fewer. However, the number of hepatic metastasis was significantly higher and the diameter was significantly larger in the group with high CE using the tumor margin as ROI. Moreover, cases with grade B or C were recognized significantly more often. Conclusion: The CE ratio correlated with the MVD. The CE ratio using the tumor margin was similar to the MVD in relation to the clinicopathological factors. Taken together, these findings suggest that the CE ratio using the tumor margin as ROI may reflect MVD.*

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In colorectal cancer, liver metastasis exists in approximately 10% of cases at initial diagnosis. Additionally, liver metastasis is the most frequently recognized as the first metastatic site after curative resection (1). In order to improve the outcome of patients with advanced colorectal cancer, it is important to control hepatic metastasis. Because curative resection of liver metastasis can lead to disease cure, surgical treatment is desirable for resectable cases (2). Since chemotherapy for metastatic colorectal cancer has progressed in recent years, conversion therapy, a widely held concept for liver metastasis in patients initially evaluated as unresectable which changes to resectable after response to chemotherapy, has been reported to improve the prognosis of patients with colorectal cancer liver metastasis (3, 4).

Since antibodies to epidermal growth factor receptor (EGFR) have the effect of early tumor shrinkage, they are often used in anticipation of conversion therapy (5-8). However, Kirsten rat sarcoma viral oncogene (*KRAS*)-mutated tumor cases cannot be treated with anti-EGFR. On the other hand, there are few contraindications to treat such cases with bevacizumab, an anti-vascular endothelial growth factor (VEGF) drug. We previously studied the effect of bevacizumab on liver metastasis using the ratio of the computed tomography (CT) value for hepatic metastatic lesions in the arterial phase in contrast-enhanced (CE) CT to that on plain CT (9). In cases with a high CE ratio, the tumor shrinkage rate was higher, whereas in those with a low CE ratio, no significant difference was noted in the tumor shrinkage rate. In addition, since the CE ratio was attenuated after treatment with bevacizumab, there was the possibility that suppression of neovascularization of the metastatic lesion by bevacizumab might reduce blood flow (9). It can be suggested that the contrast effect in liver metastatic lesions of colon cancer reflects the blood flow,

however, there is no report investigating the relation between the CT contrast effect and microvessel density (MVD) in liver metastasis. Concerning the MVD, it is reported that the MVD of the primary and metastatic sites was a factor predictive of metastasis and survival (10, 11).

In this study, the CE ratio was evaluated by two methods using the whole area of the metastasis or the lesion's margin in the preoperative CT. We analyzed the relation between the CE ratio and the MVD using the liver metastatic lesion of colorectal cancer, and examined whether the CE ratio and the MVD are related to clinicopathological factors of the primary tumor.

Patients and Methods

Patients. Thirty-two patients who underwent liver resection for metastasis from colorectal cancer excluding neoadjuvant chemotherapy cases between April 2006 and October 2011 at our Department were analyzed retrospectively.

CT imaging. CT scan was performed within 1 month before surgery for liver metastasis using a 64- or 16-row multidetector CT scanner (GE Healthcare Ltd, Hino, Japan.). CT scan was performed without contrast medium at first, followed by contrast-enhanced CT scanning during the late arterial phase (40 s) following intravenous injection with a nonionic contrast medium at 600 mgI/kg. Scanning parameters included 5 mm section thickness, a pitch of 1.375:1, and 120 kV using the Auto-mA feature.

Imaging analysis. Using the imaging control system (INFINITY-PACKS; INFINITT JAPAN Co. Ltd. Tokyo, Japan), the largest hepatic metastatic lesion was measured in each patient. The mean CT values using the lesion margin and the whole metastatic lesion were automatically evaluated. Contrast enhancement of the tumors was evaluated in terms of the ratio of the CT value during the late arterial phase to that on the plain CT (*i.e.* the CE ratio).

For the evaluation using the metastasis margin, the region of interest (ROI) was set at the same peripheral portion of the metastasis in both the late arterial phase and the plain CT. The mean CE ratio of the marginal area was obtained from three ROIs of metastasis from each patient (Figure 1). For the evaluation of the whole metastatic lesion, the outer edge of the metastatic lesion was delineated using a freehand tool on a monitor (Figure 2). The average CE values obtained from three independent investigators were used in the analysis.

Immunohistochemical analysis for MVD. Immunohistochemical analysis for MVD was carried out using an autostainer (Ventana Medical Systems Inc., Tucson, AZ, USA). Sections (4 μm) of formalin-embedded specimen of the liver metastasis were incubated with mouse anti CD31 (1:20; Dako, Glostrup, Denmark) antibody. Reaction with anti-CD31 was visualized using horseradish peroxidase conjugated secondary antibody and I-VIEW DAB Universal kit (Ventana Medical Systems, Inc.), followed by counterstaining with Carrazzi's hematoxylin solution for 1 min

Five sites of the tumor stroma of the metastasis were randomly selected. The number of microvessels stained with anti-CD30 (12, 13) was counted in a field of view at 400× and the median value was taken as the MVD (Figure 3). Since the median MVD was 13, values larger than 13 were classified as the high MVD group and values smaller than 13 were classified as the low MVD group.

Statistical analysis. JMP ver. 12.2.0 (SAS institute Inc., Cary, NC, USA) was used for statistical analysis. The correlation between two variables was evaluated using chi-square test and simple linear regression analysis. A statistically significant difference was considered at $p<0.05$.

The protocol of this study was approved by the Institutional Review Board of Tokyo Women's Medical University (approval no. 4659).

Results

Patient characteristics. The characteristics of the enrolled patients are summarized in Table I. The median age was 70 (range=33-85) years and there were 22 males and 10 females.

Assessment of CE ratio. CE ratios obtained from assessing the entire metastasis and ROI of the metastasis margin were significantly correlated by simple linear regression analysis (R2=0.4461, $p<0.0001$) (Figure 4).

MVD and method for determining the CE ratio. The MVD and the CE ratio obtained from assessing the entire metastasis were significantly correlated ($R^2=0.1539$, $p<0.001$). Additionally, the MVD and the CE ratio obtained from using ROIs of the metastasis margin were significantly correlated (R2=0.1491, $p<0.001$) (Figure 5).

Correlation of MVD with clinicopathological findings. There were no significant differences regarding gender, age, location of primary lesion, type of histology and T-category in relation to MVD. Additionally, there was no significant difference regarding extrahepatic metastasis, timing of hepatic metastasis, number of hepatic metastases and the degree of hepatic metastasis. Cases with lymphatic invasion and N2/N3 nodal status were significantly more often observed in the group with high MVD. There was also a trend for more cases with venous invasion in the group with high MVD. The maximum diameter tended to be larger in the group with high MVD, and cases with grade B or C were significantly more frequent (Table II).

Correlation of CE ratio based on the entire tumor and clinicopathological findings. When patients were divided by the median value into high CE and low CE groups, there was no significant difference regarding gender, age, location of primary lesion, type of histology, T-category, lymphatic invasion, venous invasion and nodal status. There was also no significant difference regarding extrahepatic metastasis, timing of hepatic metastasis, and the degree of hepatic metastasis. In the group with high CE, there were significantly fewer cases with grade B or C. Additionally, the

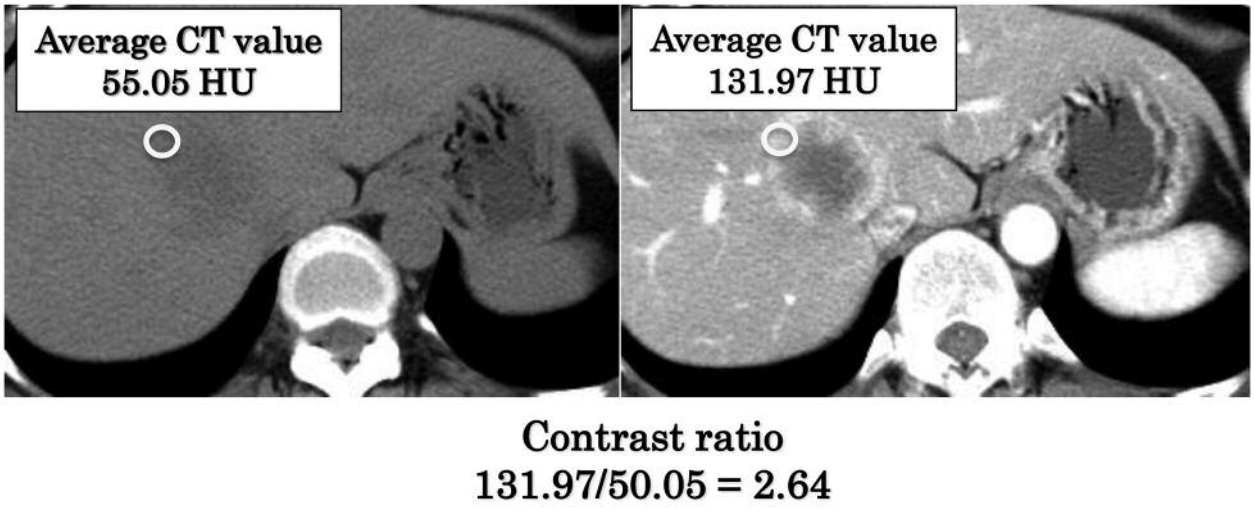

Figure 1. *For the evaluation of the marginal area of the metastasis, regions of interest (ROIs) were set at the same peripheral portion of the metastasis in both the late arterial phase and the plain computed tomography (CT). The mean CT ratio of the marginal area was obtained using three ROIs of metastasis from each patient.*

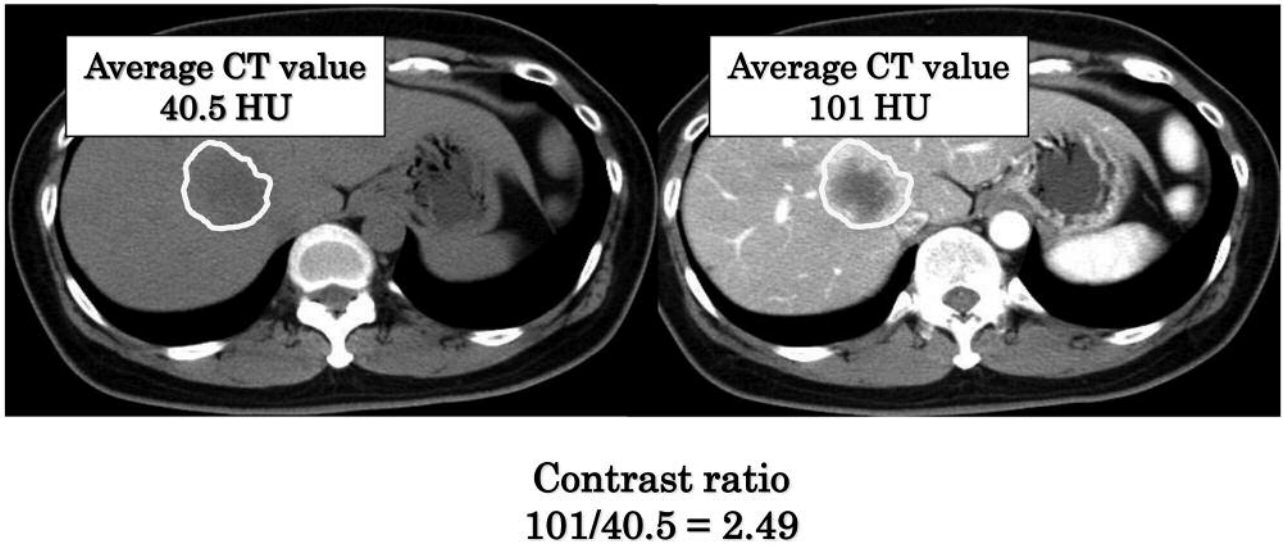

Figure 2. *For the evaluation of the whole metastatic lesion by computed tomography (CT), the outer edge of the metastatic lesion was surrounded using a freehand tool on a monitor.*

number of hepatic metastases was significantly higher in the group with high CE and the diameter tended to be larger (p=0.0799); cases with metastasis diameter of 25 mm or more were more frequent, and cases with grade B or C tended to be more frequent (Table III).

Correlation of CE ratio based on the tumor margin with clinicopathological findings. When the CE ratio was divided by the median value into high CE and low CE groups, there was no significant difference regarding gender, age, location of the primary lesion, type of histology, T-category, lymphatic invasion, venous invasion, node status, extrahepatic metastasis, timing of hepatic metastasis and the degree of hepatic metastasis. The number of hepatic metastases was significantly higher in the high CE group and the diameter was significantly larger; cases with grade B or C were significantly more frequent (Table IV).

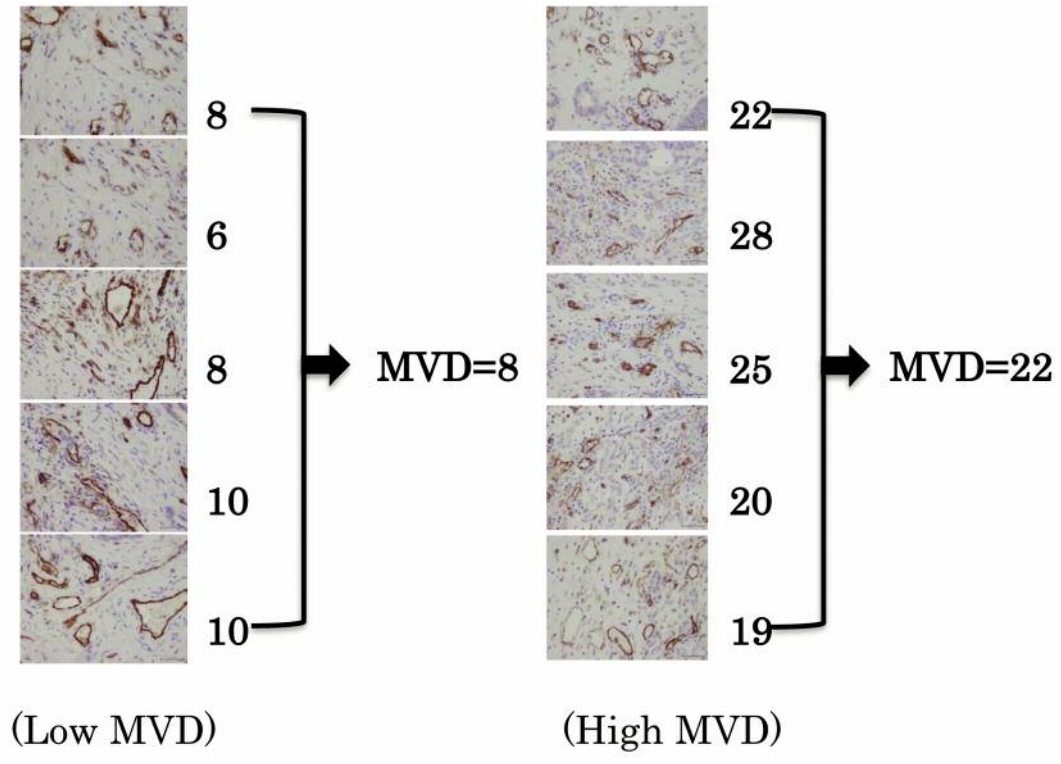

Figure 3. *Tumor stroma of the metastasis was randomly selected from five sites. The number of microvessels stained with anti-CD 31 was counted in a field of view at 400× and the median value was taken as the microvessel density (MVD).*

Discussion

Specific binding of bevacizumab to human VEGF inhibits binding of VEGF to VEGF receptor on vascular endothelial cells (15-17). During progression and proliferation of tumor *in vivo*, especially colon cancer cases, VEGF production is known to be enhanced following the increase of neovascularization. Inhibiting the bioactivity of VEGF by bevacizumab inhibits tumor growth *via* inhibition of angiogenesis or normalization of the immature and incomplete blood vessels induced by VEGF from tumors (17-20). During chemotherapy combined with bevacizumab for patients with colorectal cancer patients, liver metastatic lesions often show a cystic change in the CT image which indicates an antitumor effect without shrinkage. Patients with complete cystic changes are reported to have a favorable prognosis (22). These findings support the fact that tumor necrosis is induced by the blockade of blood flow by bevacizumab. Our previous report indicated that the CE ratio in the metastatic lesion in the liver decreased after chemotherapy induction combined with bevacizumab in patients with colon cancer liver metastasis (9). This observation also suggested that a decrease of CE ratio in the liver metastatic lesion indicates reduced blood flow in the tumor area due to inhibition of angiogenesis by bevacizumab induced in the tumor cells. Therefore, this study was conducted to investigate the correlation between the CT contrast effect and MVD using the cases which underwent resection of liver metastases.

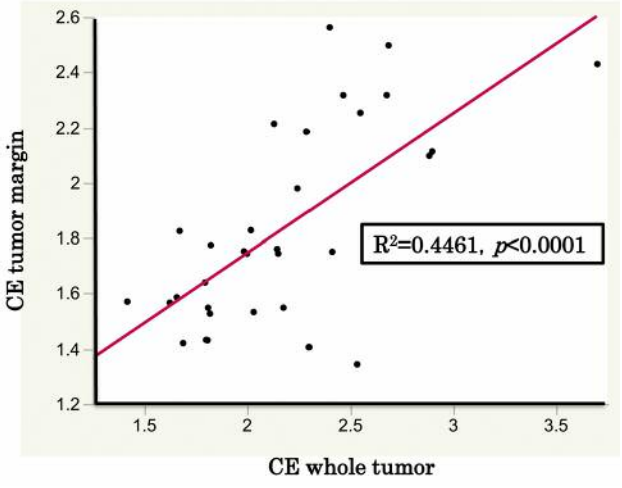

Figure 4. *Correlation of contrast enhancement (CE) ratios obtained from assessment using the whole tumor and tumor margin as regions of interest.*

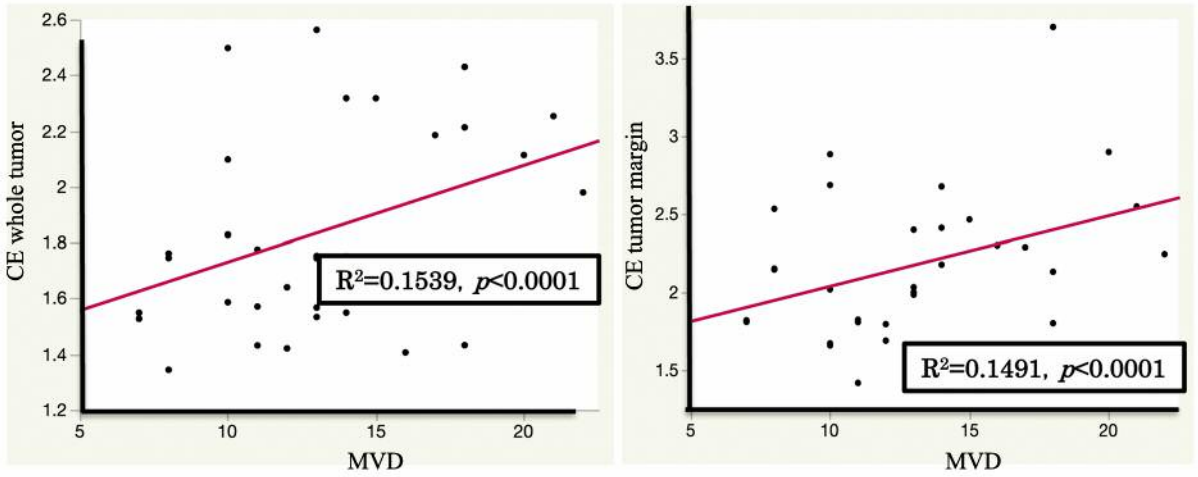

Figure 5. *Correlation of microvessel density (MVD) and contrast enhancement (CE) ratios obtained from assessment using the whole tumor and tumor margin as regions of interest.*

Table I. *Patient background characteristics.*

Characteristic	Value
Age, years	
Median (range)	70 (33-85)
Gender, n	
Male	22
Female	10
Site of disease, n	
Colon	26
Rectum	6
Size of metastasis, mm	
Median (range)	25.2 (13-94.6)
Degree , n*	
H1	28
H2	2
H3	2
Grade, n*	
A	21
B	7
C	4
Timing of hepatic metastasis, n	
Synchronous	15
Metachronous	17

*According to Japanese Classification of Colorectal Carcinoma (14).

FirstIy, when the evaluation of CE ratio using the marginal region or the whole metastatic lesion was carried out, a significantly positive correlation was found between the evaluation methods. Additionally, for both methods, the CE ratio significantly positively correlated with MVD in the metastatic lesion. Taken together, it could be considered that the effect of the CT contrast enhancement reflects the blood vessel density of the tumor. In other words, if the high contrast effect represents a high MVD induced by VEGF, the efficacy of bevacizumab against a metastatic lesion with high CE might be predicted.

Generally, primary lesions associated with high MVD or high VEGF tend to metastasize, and there tends to be similar characteristics in the metastatic lesion (10, 11). In this study, the relation between MVD, CT contrast enhancement and clinocopathological factors were investigated. In the group with high MVD for the liver metastatic lesion, cases with lymphatic invasion and venous invasion of the primary site were significantly more than frequent than in the low MVD cases. There tended to be more lymph node-positive cases found in those with high MVD. In addition, cases with larger metastatic tumor diameters and more metastatic nodules had higher MVD in their metastatic lesions. These findings were also shown in the cases with CE ratio evaluated using the marginal region. However, the relation was dissimilar in the cases with CE ratio evaluated using the whole metastatic tumor. Although the CE ratio correlated with the MVD regardless of whether it was evaluated using the margin or the whole metastatic lesion, the evaluation of contrast enhancement at the margin might be better than that of the whole metastatic lesion in the prediction of the MVD based on the compatibility with the clinicopathological factors. This might be explained by the fact that compared with using the lesion margin, evaluating an entire metastasis includes a wide area of necrosis, which may disproportionally affect the evaluation of MVD.

Konerding *et al*. reported that the intervessel distance was shorter in primary colorectal tumors which had already metastasized with compared to those without metastases at the time of surgery (22). In other words, the MVD was high in primary tumors with metastasis. If the MVD is higher in

Table II. *Clinicopathological factors according to microvessel density (MVD).*

Factor	High MVD	Low MVD	*p*-Value
Gender, n			
Male	11	11	0.712
Female	6	4	
Age, years			
Median (range)	70 (33-82)	70 (36-85)	0.5969
Site of disease			
Colon	14	12	0.9999
Rectum	3	3	
Differentiation, n			
Well, moderate	16	14	0.4839
Poorly, mucinous	0	1	
Tumor depth, n			
T3	12	14	0.3326
T4	4	1	
Lymphatic invasion, n			
Positive	11	4	**0.0106**
Negative	4	11	
Venous invasion, n			
Positive	9	4	0.0654
Negative	6	11	
Nodal status, n			
N0, N1	10	14	**0.0402**
N2, N3	6	1	
Extrahepatic metastasis, n			
Positive	2	3	0.6454
Negative	15	12	
Timing of hepatic metastasis, n			
Simultaneous	9	6	0.7061
Metachronous	8	9	
Number of hepatic metastases, n			
Single	11	12	0.444
Multiple	6	3	
Size of metastasis, mm			
Median	29.5	21	0.0813
≥25 mm	11	6	0.2971
<25 mm	6	9	
Degree, n*			
≥H1	14	14	0.6029
>H2	3	1	
Grade of liver metastasis, n*			
A	8	13	**0.0186**
B, C	9	2	

*According to Japanese Classification of Colorectal Carcinoma (14). Values shown in bold are significantly different ($p<0.05$).

Table III. *Clinicopathological factors according to the contrast enhancement ratio (CE) based on evaluation of whole metastases.*

Factor	High CE	Low CE	*p*-Value
Gender			
Male	11	11	0.712
Female	6	4	
Age, years			
Median (range)	70 (33-85)	70 (36-82)	0.8205
Site of disease			
Colon	15	11	0.3828
Rectum	2	4	
Differentiation, n			
Well, moderate	17	13	0.4516
Poorly, mucinous	0	1	
Tumor depth, n			
T3	14	12	0.9999
T4	3	2	
Lymphatic invasion, n			
Positive	8	7	0.9999
Negative	9	6	
Venous invasion, n			
Positive	9	4	0.2828
Negative	8	9	
Nodal status, n			
N0, N1	12	12	0.4117
N2, N3	5	2	
Extrahepatic metastasis, n			
Positive	1	4	0.1609
Negative	16	11	
Timing of hepatic metastasis, n			
Simultaneous	8	9	0.7061
Metachronous	9	6	
Number of hepatic metastases, n			
Single	11	12	0.4440
Multiple	6	3	0.2956
Size of metastasis, mm			
Median	29.1	20.4	
≥25 mm	11	6	0.2971
<25 mm	6	9	
Degree, n*			
≥H1	17	11	0.1571
>H2	0	4	
Grade of liver metastasis, n*			
A	12	9	**0.0186**
B, C	5	6	

*According to Japanese Classification of Colorectal Carcinoma (14). Values shown in bold are significantly different ($p<0.05$).

a metastasis, the prediction of the effect of bevacizumab on metastatic tumor might be judged by the degree of CT contrast enhancement. Strohmeyer *et al*. also reported that the expression of VEGF and MET proto-oncogene receptor tyrosine kinase (MET) in prostate cancer mainly affected the progression of tumor through an effect on MVD (23). This report suggested that a similar result can be obtained, not only in colon cancer, but also in other cancer types.

The recent development of dual energy CT has been reported to capture images using different voltages and evaluate the MVD at the target region using the assessment of iodine uptake (24-26). The use of dual energy CT might be able to evaluate the blood flow of liver metastasis from colorectal cancer more precisely than single-energy CT. Future studies utilizing an analysis similar to our report will be needed concerning the methods of MVD assessment,

Table IV. *Clinicopathological factors to the contrast enhancement ratio (CE) based on evaluation of the marginal region.*

Factor	High CE	Low CE	*p*-Value
Gender			
Male	11	11	>0.99
Female	5	5	
Age, years			
Median (range)	73 (33-85)	68.5 (36-82)	0.2954
Site of disease			
Colon	14	12	0.6539
Rectum	2	4	
Differentiation, n			
Well, moderate	16	15	0.9999
Poorly, mucinous	0	1	
Tumor depth, n			
T3	15	11	0.1719
T4	1	4	
Lymphatic invasion, n			
Positive	10	5	0.0679
Negative	5	10	
Venous invasion, n			
Positive	9	4	0.0654
Negative	6	11	
Nodal status, n			
N0, N1	10	14	0.2200
N2, N3	5	2	
Extrahepatic metastasis, n			
Positive	1	4	0.3326
Negative	15	12	
Timing of hepatic metastasis, n			
Simultaneous	8	7	0.9999
Metachronous	8	9	
Number of hepatic metastases, n			
Single	8	15	**0.0059**
Multiple	8	1	**0.0204**
Size of metastasis, mm			
Median	31.3	20.6	
≥25 mm	12	5	**0.0131**
<25 mm	4	11	
Degree, n*			
≥H1	13	15	0.5996
>H2	3	1	
Grade of liver metastasis, n*			
A	8	13	0.0627
B, C	8	3	

*According to Japanese Classification of Colorectal Carcinoma (14). Values shown in bold are significantly different ($p<0.05$).

blood flow and the prediction of the antitumor effect of bevacizumab.

Conclusion

The CE ratio correlated with MVD. Although evaluation methods using the entire tumor and the tumor margin both correlated with the MVD, the CE ratio using the tumor margin was similar to MVD in its relationship with the clinicopathological factors. Taken together, these findings may suggest that the CE ratio using the tumor margin possibly reflects the MVD.

Conflicts of Interest

The Authors had no conflict of interest in regard to this study. No financial or material support was received for this work.

Authors' Contributions

YY, KY and MS performed the assessment of CE ratio and analyzed the relations to the clinicopathological factors. YY and KY prepared the manuscript. HY and MS supported the collection of the patient's data. HI performed immunohistochemical analysis. YN supervised all of this work.

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