

## Significance of the Difference in the Size of Liver Tumors in the Management of Patients with Colorectal Liver Metastases

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### Abstract

**Background:** The combination of chemotherapy and surgery is currently accepted for the treatment of patients with technically resectable colorectal liver metastases. It is, however, hard to determine which of these modalities should be the forward treatment. In this study, we assessed the usefulness of the difference in tumor size assessed in pretherapeutic imaging in the selection of chemotherapy in these patients.

**Methods:** We present a retrospective review of 80 consecutive colorectal liver metastases without extrahepatic tumors. The relapse-free survival (RFS), progression-free survival (PFS) and overall survival (OS) were evaluated and compared between patients who underwent surgery (n=66) and chemotherapy (n=14) according to clinical features. In particular, we addressed pretherapeutic imaging studies including the distribution and number of metastatic liver tumors. In addition, the ratio of tumor size (largest to smallest tumor) was calculated; two groups classified as  $R < 5$  (ratio  $< 5$ ) and  $R \geq 5$  (ratio  $\geq 5$ ) were compared.

**Results:** Univariate analysis was performed in the surgery group; significant differences in RFS were found regarding time of occurrence, the number of tumors and the ratio of tumor diameters. Multivariate analysis showed that the ratio of tumor size,  $R \geq 5$ , was the only independent prognostic risk factor concerning both RFS and OS. We then compared the outcome of patients with prognostic risk factors between surgery and chemotherapy. Surgery achieved significantly better OS than chemotherapy, with the exception of the  $R \geq 5$  group. No difference in OS, in addition to RFS and PFS, was seen in the  $R \geq 5$  groups regardless of treatment.

**Conclusion:** Colorectal cancer patients with resectable liver metastases with  $R \geq 5$  showed no significant difference in outcome using surgery or chemotherapy. Chemotherapy could be used as an alternative to forward surgery to address oncological concerns such as the presence of latent metastases or poor treatment outcome in these patients.

**Keywords:** Colorectal liver metastasis; Oncological prognostic factors; Ratio of tumor size; Colon cancer; Pre-therapeutic imaging

### Introduction

The liver is the most common site of distant metastases in patients with colorectal cancer (CRC). Hepatic resection for CRC liver metastases is the only potential curative treatment, with a reported 5-year survival rate in the range of 36% to 61% [1-3]. R0 curative resection can be carried out for patients with resectable liver metastases regardless of tumor number [4]. However, recurrences have been reported in two-thirds of patients, half occurring in the residual liver after curative liver resection [5-7]. Persistence of latent metastases is the most likely explanation for recurrence, which probably leads to relapse within a short time period following surgery. Oncological concerns remain even for technically resectable liver metastases. Resectable CRC liver metastases should be assessed from both a technical and an oncological viewpoint.

Recent improvements in chemotherapy have been shown to prolong the survival of CRC patients with unresectable liver metastases. Combination regimens using various biological agents in combination with cytotoxic chemotherapy have achieved high response rates and reduced tumor size [8], which renders technically unresectable liver metastases resectable. In addition to unresectable liver metastases, chemotherapy has been attempted for resectable liver metastases to treat occult metastases and control tumor progression in Europe [9]. The European Colorectal Metastases Treatment Group have recommended preoperative chemotherapy for all patients with CRC liver metastases [10] because of the evidence that preoperative chemotherapy prolongs progression-free survival (PFS) in patients with resectable liver

metastases. The combination of chemotherapy and surgery is currently accepted for the treatment of patients with technically resectable colorectal liver metastases. However, the impact of preoperative chemotherapy on overall survival (OS) remains unclear [11]. Therefore, it is debatable as to whether chemotherapy or surgery should be used as the forward treatment of resectable CRC liver metastases; it has not been established which CRC patients with resectable liver metastases require chemotherapy before surgery. The oncological behavior of tumors should be considered to determine patient outcome including the occurrence of latent metastases in preoperative imaging studies. Several studies have reported prognostic risk factors that can be used to predict malignant potential, such as the number, size and distribution of the liver tumors; however, there is no consensus regarding how to apply these characteristics to determine whether chemotherapy is indicated [6,12-24].

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In the present study, we evaluated the effectiveness of different sized tumors as an indicator of oncological behavior. A mixture of small and large tumors may represent the possible existence of latent metastases in pretherapeutic imaging, but no study has been attempted to evaluate its significance. We assessed its impact on the selection of CRC patients with resectable liver metastases who need chemotherapy before surgery.

Materials and Methods

Patients

Eighty consecutive CRC patients with liver tumors were recruited in this retrospective study. Sixty-six patients underwent hepatic resection and 14 had chemotherapy for initial treatment of liver metastases between November 2005 and September 2012 at the Saitama Medical Center, Jichi Medical University. Surgery was selected for patients harboring safe residual liver volume with no evidence of vascular invasion requiring vascular anastomosis other than portal vein; otherwise, patients underwent chemotherapy. CRC patients with both synchronous and metachronous liver metastases were included, but patients with extrahepatic metastases were excluded. To assess extrahepatic disease, all patients underwent ultrasonography, enhanced CT and enhanced MRI or CT angiography, and chest CT and colonoscopy. Intraoperative ultrasonography was carried out to detect preoperative unknown tumors and to guide resection in patients who underwent hepatic resection. This study was approved by the Research Ethics Committees of Jichi Medical University. Written informed consent was obtained from each study participant.

Assessment

Relapse-free survival (RFS) and OS were assessed in 66 patients in the surgery group, and compared in relation to several factors including time of occurrence (synchronous or metachronous), primary tumor locations, levels of the tumor marker, tumor distribution, number of tumors, and difference in tumor size. For comparison of the difference in distribution, unilateral and bilateral metastases were compared. The difference was assessed between patients with <5 tumors (N<5) and patients with ≥ 5 tumors (N ≥ 5). The cutoff value, N=5, was determined as had been referred to be a prognostic factor in the several literatures [25]. In addition, the ratio of size regarding the largest to smallest tumor

Variables	Surgery n=66	Chemotherapy n=14	p value
Age, Mean	63.8 ± 10.9	63.4 ± 6.5	NS
Gender			
Male	45	9	NS
Female	21	5	
Diagnosis of liver metastases			
Synchronous	27	13	0.022
Metachronous	39	1	--
Site of primary tumor			
Right Colon	15	5	NS
Left Colon	21	5	
Rectum	30	4	
Tumor distribution			
Unilateral	44	2	<0.001
Bilateral	22	12	--
Number of tumors			
1-4	51	3	<0.001
5-8	11	5	
>8	4	6	
Largest tumor diameter (cm)			
<5	56	11	NS
≥ 5	10	3	
Ratio of tumor diameter: Largest tumor/smallest tumor			
<5	58	7	0.003
≥ 5	8	7	--
Pre-therapeutic CEA level (ng/ml), median	8.1	109.3	<0.001
CEA: Carcino-Embryonic Antigen			

Table 1: Patient characteristics in the surgery and chemotherapy groups.

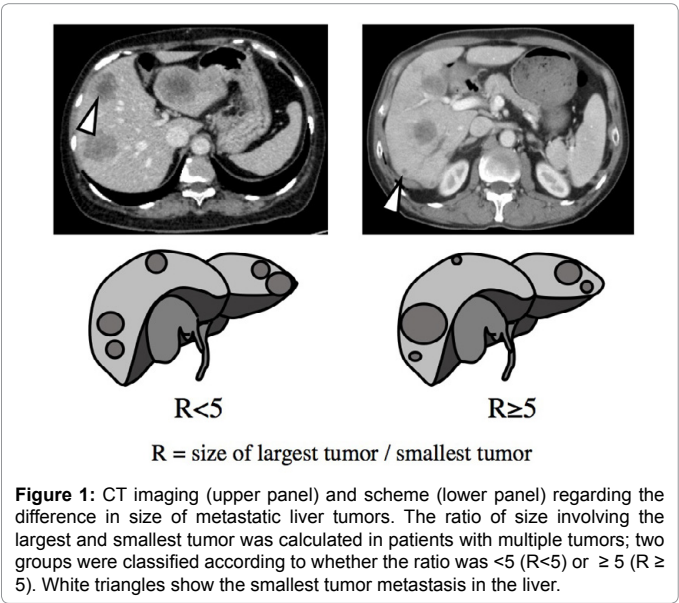
was calculated; size ratios <5 (R<5) and ≥ 5 (R ≥ 5) were evaluated to compare the difference concerning tumor size (Figure 1). PFS and OS were also assessed in 14 patients in the chemotherapy group, and then compared with those in the surgery group.

Statistical analysis

Fisher's exact test was used to examine the relationship between two categorical variables. Continuous comparisons of variables between the two groups were performed; Student's t-test was used for those variables that followed a normal distribution, and the non-parametric Mann-Whitney-Wilcoxon test was used for those variables that did not follow a normal distribution. A multivariate Cox regression analysis was performed to identify significant contributors that were independently associated with OS and RFS or PFS among those factors. The level of statistical significance was set at p<0.05. Values are shown as the mean ± SE. OS and RFS or PFS data were plotted as Kaplan-Meier curves, and the differences among the groups were compared using the log-rank test. We used StatView version 5.0 (SAS institute Inc., Cary, NC) for the statistical analysis.

Results

In the surgery group, hemihepatectomy or more extensive resection was performed in 15 (22.7%) patients, segmentectomy in seven (10.6%) and non-anatomic partial hepatectomy in 44 (66.7%). Curative R0 resection was performed in 61 (92.4%) patients; however, histologically five (7.6%) patients exhibited cancer cells in the surgical margin of the resected specimen. 27 (40.9%) patients had synchronous liver metastases, 11 of whom underwent simultaneous resection with the primary tumor. The mortality rate was 1.5% and morbidity rate was 25.8%. One patient died as a result of bowel leakage at the time of simultaneous surgery involving the primary tumor. On the basis



of the benefit of perioperative chemotherapy reported in 2008 [26], adjuvant chemotherapy was given to all the patients who underwent liver resection due to metastatic CRC in our hospital since 2009, which accounted for 36 (54.5%) patients. In the chemotherapy group, a 5FU + oxaliplatin-based regimen was administered in 13 patients, and an irinotecan + cisplatin regimen was performed in one patient. The anti-VEGF antibody, bevasizumab, was administered in six patients, and the anti-EGFR antibodies (either panitumumab or cetuximab) were used in three patients. Three patients (21.4%) underwent conversion surgery for liver metastases after the chemotherapy.

The characteristics of the 80 patients are detailed in (Table 1); 66 patients in the surgery group were compared with 14 patients in the chemotherapy group. Several cutoff values rather than  $R \geq 5$  ( $R \geq 2, 3, 4, 6$ ) were applied to the comparison of RFS in the surgery group. Regardless of which cutoff value was adopted, RFS was significantly worse in the high R group ( $p = 0.014, p = 0.097, p = 0.004, p < 0.001, p < 0.001$  for  $R \geq 2, 3, 4, 5, 6$ , respectively). Among these cut off values, multivariate stepwise Cox regression analysis identified  $R \geq 5$  as the most contributor to affect RFS. Therefore,  $R \geq 5$  was determined as the representative of the value of the difference in tumor size. Thirty-six patients with adjuvant chemotherapy in 66 patients in the surgery group showed significant longer RFS than those without (24.1 months vs. 6.3 months, respectively;  $p = 0.011$ ) but no difference was seen in comparison of OS (40.8 months vs. 49.5 months, respectively;  $p = 0.994$ ). No significant difference of the number of patients with and without adjuvant chemotherapy was seen between synchronous and metachronous, unilateral and bilateral,  $N \geq 5$  and  $N < 5$ , and  $R \geq 5$  and  $R < 5$  ( $p = 0.891, p = 0.292, p = 0.915, p = 0.302$ , respectively), which indicated that adjuvant chemotherapy did not affect the comparison of RFS and OS in several predictive factors.

No significant difference was seen in age, gender, primary tumor location, and largest diameter of tumors between surgery and chemotherapy; however, a significant difference was observed regarding

Variables	Number of cases	Median RFS (months)	p value*	Median OS (months)	p value*
Diagnosis of liver metastases					
Synchronous	27	12.2	0.015	42.2	0.532
Metachronous	39	26.8	--	46.7	--
Site of primary tumor					
Right Colon	15	30.5	--	50.1	--
Left Colon	21	21.3	0.620	47.9	0.571
Rectum	30	16.6	--	42.1	--
Pre-therapeutic CEA level (ng/ml)					
<5.0	32	31.2	0.398	49.9	0.653
$\geq 5.0$	33	16.6	--	39.9	--
Unknown	1	--	--	--	--
Tumor distribution					
Unilateral	44	21.4	0.084	49.1	0.727
Bilateral	22	14.5	--	44.4	--
Number of tumors					
<5	51	26.8	0.001	47.8	0.252
$\geq 5$	15	9.9	--	42.0	--
Ratio of tumor diameter: Largest tumor/smallest tumor					
<5	58	21.4	<0.001	47.3	0.001
$\geq 5$	8	3.7	--	32.9	--

\* Log-rank test  
RFS: Relapse Free Survival; OS: Overall Survival; CEA: Carcino Embryonic Antigen

Table 2: Univariate analysis of predictive factors of treatment outcome in the 66 cases in the surgery group.

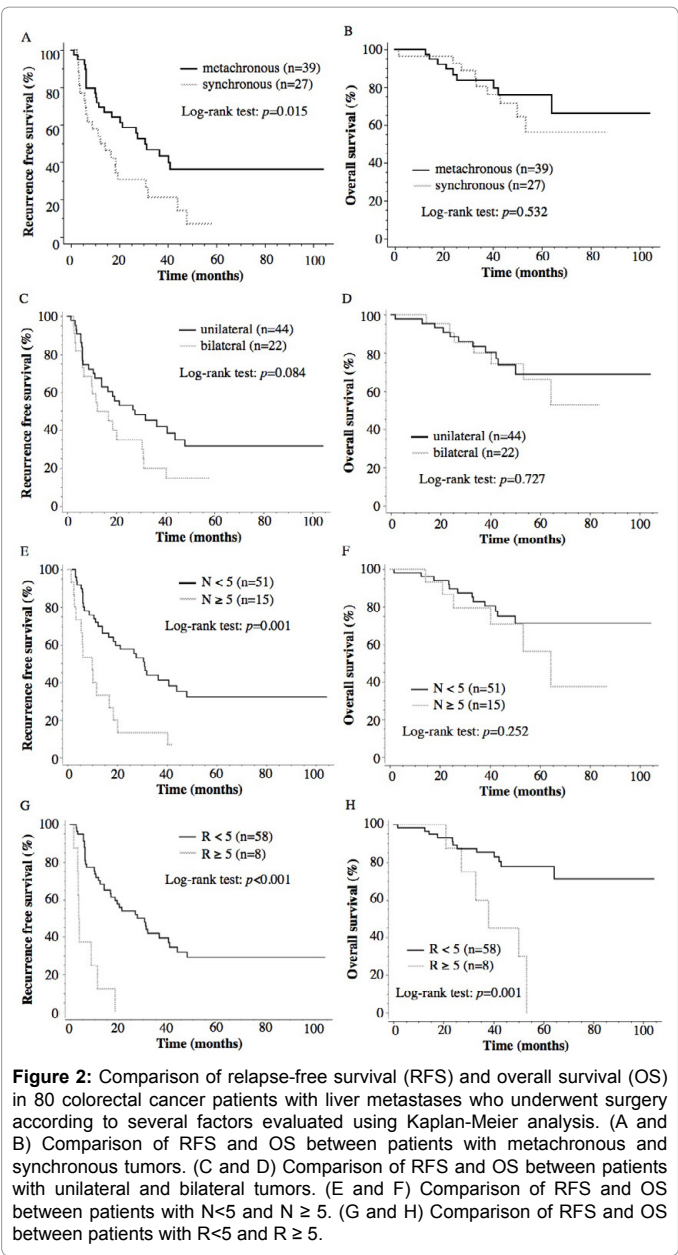


Figure 2: Comparison of relapse-free survival (RFS) and overall survival (OS) in 80 colorectal cancer patients with liver metastases who underwent surgery according to several factors evaluated using Kaplan-Meier analysis. (A and B) Comparison of RFS and OS between patients with metachronous and synchronous tumors. (C and D) Comparison of RFS and OS between patients with unilateral and bilateral tumors. (E and F) Comparison of RFS and OS between patients with  $N < 5$  and  $N \geq 5$ . (G and H) Comparison of RFS and OS between patients with  $R < 5$  and  $R \geq 5$ .

time of occurrence, tumor distribution, the number of tumors, the difference in size of tumors and tumor markers between surgery and chemotherapy. In contrast to patients in the surgery group, patients in the chemotherapy group had several poor prognostic risk factors. To address the association between size of the liver tumor and its localization in patients' liver, we compared size of the liver tumor between right and left hepatic lobe in 31 patients with single liver metastasis. No significant difference of size of the liver tumor was seen between them ( $p = 0.941$ ). In addition, no significant difference was seen between 4 segments of the liver, anterior segment of right hepatic lobe, posterior segment of right hepatic lobe, medial segment of left hepatic lobe and lateral segment of left hepatic lobe ( $p = 0.862$ ). Univariate analysis was performed to elucidate the impact of these factors on the outcome of RFS and OS in the surgery group (Table 2). This analysis revealed a significant difference in RFS concerning time of occurrence, the number of tumors present and the ratio of tumor diameters ( $p = 0.015, p = 0.001, p < 0.001$ , respectively; Table 2). However, a significant difference in OS

was observed only regarding the ratio of tumor diameters ( $p=0.001$ , Table 2). Comparison of survival curves for RFS and OS according to several factors such as time of occurrence, tumor distribution, the number of tumors and difference in tumor size are presented in (Figure 2). To independently verify prognostic factors affecting the outcome, we chose time of occurrence, the number of tumors present and the ratio of tumor diameters for multivariate analysis. In addition, tumor distribution was also included because it can be a prognostic factor that can be estimated in imaging studies before surgery as well as other parameters. Multivariate analysis revealed that the ratio of tumor size was the only independent prognostic factor regarding both RFS and OS (Table 3). To assess malignant potential, such as recurrence in the remnant liver, we focused on only liver metastasis after surgery, suggesting the liver recurrence occurred significant frequently in the patients with synchronous metastases and  $R \geq 5$ , but not in other risk factor patients (Table 4).

Next, we compared the impact of surgery with that of chemotherapy on outcome. To elucidate the significance of surgery, time to relapse regarding RFS in the surgery group was compared with time to progression regarding PFS in the chemotherapy group. There was a significant difference between them; the median RFS was 14.5 months in the surgery group versus a median PFS of 7.0 months in the chemotherapy group ( $p=0.001$ ). The surgery group achieved a significantly better OS than the chemotherapy group; the median OS was 46.3 months in the surgery group versus 22.1 months in the chemotherapy group ( $p=0.001$ ). Comparison of survival curves between RFS in the surgery group and PFS in the chemotherapy group is presented in (Figure 3A). Comparison of the survival curves for OS between the surgery group and the chemotherapy group is presented in (Figure 3B).

In consideration of the important prognostic risk factors such as time of recurrence, tumor distribution, the number of tumors and the ratio of tumor diameters, we compared the outcome between the surgery group and the chemotherapy group. Features involving the presence of synchronous tumors, bilateral distribution,  $N \geq 5$  and  $R \geq 5$  were associated with a poorer prognosis concerning RFS and PFS than those involving the presence of metachronous tumors, unilateral distribution,  $N < 5$  and  $R < 5$ . RFS in the surgery group and PFS in the chemotherapy group were compared in patients with these prognostic risk factors.

Median RFS in the surgery group and PFS in the chemotherapy group, and OS between the surgery group and the chemotherapy group were compared (Table 5) in consideration of several prognostic risk factors: bilateral tumors, number of liver metastases  $\geq 5$  ( $N \geq 5$ ) and  $R \geq 5$ . Patients with synchronous tumors in the surgery group achieved better RFS than PFS in the chemotherapy group, but the difference was not significant (12.2 versus 8.4 months, respectively;  $p=0.144$ ). Patients with bilateral tumors in the surgery group had a significantly better RFS than PFS in the chemotherapy group (14.5 and 7.0 months, respectively;  $p=0.035$ ). Patients with  $N \geq 5$  in the surgery group achieved a better RFS than PFS in the chemotherapy group, but the difference was not significant (9.9 and 8.2 months, respectively;  $p=0.699$ ). Patients with  $R \geq 5$  in the surgery group achieved a poorer RFS than PFS in the chemotherapy group, but the difference was not significant (3.7 and 5.8 months, respectively;  $p=0.243$ ). There was no significant difference between the surgery and chemotherapy groups regarding prognostic risk factors, with the exception of bilateral metastases. Comparisons of survival curves for the RFS in the surgery group and PFS for the chemotherapy group in consideration of several prognostic risk factors are presented in Figures 3C, 3E, 3G and 3I.

In comparing patients with these prognostic risk factors, those with synchronous metastases in the surgery group achieved a significantly better OS than those in the chemotherapy group (46.7 and 22.0 months, respectively;  $p=0.013$ ). Patients with bilateral metastases in the surgery group had a significantly better OS than those in the chemotherapy group (49.1 and 19.1 months, respectively;  $p=0.003$ ). In addition, patients with  $N \geq 5$  in the surgery group displayed significantly better OS than those in the chemotherapy group (42.0 and 16.2 months, respectively;  $p=0.017$ ). However, patients with  $R \geq 5$  did not exhibit a significant difference in OS between surgery and chemotherapy (32.9 and 10.5 months, respectively;  $p=0.610$ ). Comparisons of the survival curves for OS between the surgery group and the chemotherapy group in consideration of several prognostic risk factors are presented in (Figures 3D, 3F, 3H and 3J).

In the surgery group, hemihepatectomy or more extensive resection was performed in 15 (22.7%) patients, segmentectomy in seven (10.6%) and non-anatomic partial hepatectomy in 44 (66.7%). Curative R0 resection was performed in 61 (92.4%) patients; however, histologically five (7.6%) patients exhibited cancer cells in the surgical margin of the resected specimen. 27 (40.9%) patients had synchronous liver metastases, 11 of whom underwent simultaneous resection with the primary tumor. The mortality rate was 1.5% and morbidity rate was 25.8%. One patient died as a result of bowel leakage at the time of simultaneous surgery involving the primary tumor. On the basis of the benefit of perioperative chemotherapy reported in 2008 [26], adjuvant chemotherapy was given to all the patients who underwent liver resection due to metastatic CRC in our hospital since 2009, which

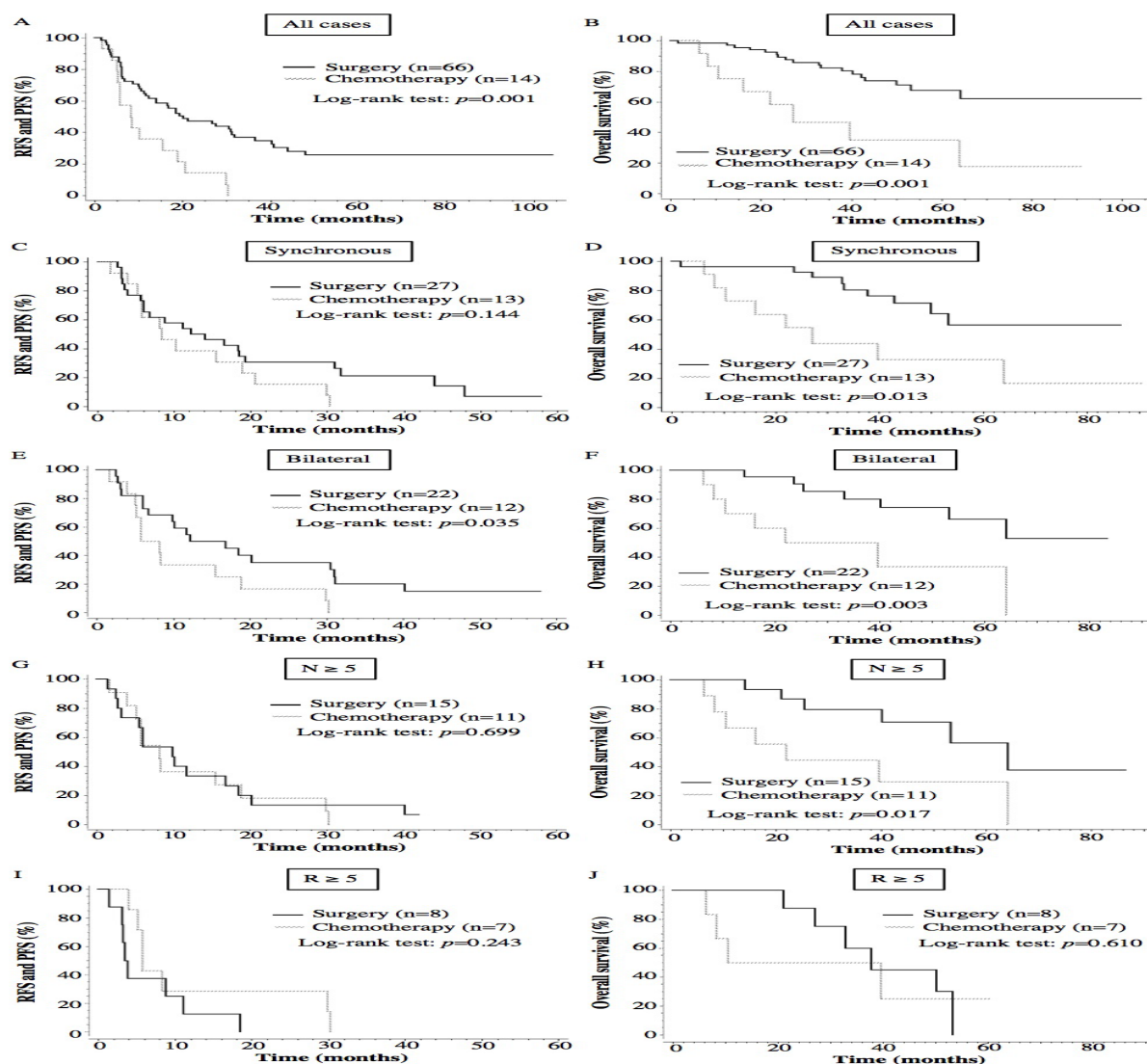
RFS			
Factor	Hazard ratio	95% CI	p-value
Synchronous metastases	1.47	0.76–2.87	0.256
Bilateral metastases	0.77	0.30–2.02	0.598
$N \geq 5$	2.85	0.99–8.17	0.051
$R \geq 5$	3.36	1.32–8.56	0.011
OS			
Factor	Hazard ratio	95% CI	p-value
Synchronous metastases	0.65	0.20–2.14	0.481
Bilateral metastases	0.92	0.24–3.55	0.899
$N \geq 5$	1.37	0.33–5.75	0.669
$R \geq 5$	5.35	1.43–20.02	0.013

CI: Confidence Interval; RFS: Relapse Free Survival; PFS: Progression Free Survival; OS: Overall Survival

**Table 3:** Multivariate analysis concerning the prediction of treatment outcome in the surgery group.

Variables	Liver recurrence		p value
	Present	Absent	
Diagnosis of liver metastases			
Synchronous	18	16	0.040
Metachronous	9	23	--
Tumor distribution			
Unilateral	20	24	NS
Bilateral	14	8	
Number of tumors			
<5	24	27	NS
≥ 5	10	5	
Ratio of tumor diameter: Largest tumor/smallest tumor			
<5	27	31	0.022
≥ 5	7	1	--

**Table 4:** Frequency of liver recurrence regarding each prognostic factor in the surgery group.



**Figure 3:** Comparison of relapse-free survival (RFS) in the surgery group (n=66) with progression-free survival (PFS) in the chemotherapy group (n=14) and comparison of OS between the surgery group and the chemotherapy group in patients with prognostic risk factors. (A) Comparison of RFS with PFS in all patients. (B) Comparison of OS between surgery and chemotherapy in all patients. (C) Comparison of RFS with PFS in patients with synchronous tumors. (D) Comparison of OS in patients with synchronous tumors between surgery and chemotherapy. (E) Comparison of RFS with PFS in patients with bilateral tumors. (F) Comparison of OS in patients with bilateral tumors between surgery and chemotherapy. (G) Comparison of RFS with PFS in patients with  $N \geq 5$ . (H) Comparison of OS in patients with  $N \geq 5$  between surgery and chemotherapy. (I) Comparison of RFS with PFS in patients with  $R \geq 5$ . (J) Comparison of OS in patients with  $R \geq 5$  between surgery and chemotherapy.

accounted for 36 (54.5%) patients. In the chemotherapy group, a 5FU + oxaliplatin-based regimen were administered in 13 patients, and an irinotecan + cisplatin regimen was performed in one patient. The anti-VEGF antibody, bevasizumab, was administered in six patients, and the anti-EGFR antibodies (either panitumumab or cetuximab) were used in three patients. Three patients (21.4%) underwent conversion surgery for liver metastases after the chemotherapy.

The characteristics of the 80 patients are detailed in (Table 1); 66 patients in the surgery group were compared with 14 patients in the chemotherapy group. Several cutoff values rather than  $R \geq 5$  ( $R \geq 2, 3, 4, 6$ ) were applied to the comparison of RFS in the surgery group. Regardless of which cutoff value was adopted, RFS was significantly worse in the high R group ( $p=0.014, p=0.097, p=0.004, p<0.001, p<0.001$  for  $R \geq 2, 3, 4, 5, 6$ , respectively). Among these cut off values, multivariate stepwise Cox regression analysis identified  $R \geq 5$  as the

most contributor to affect RFS. Therefore,  $R \geq 5$  was determined as the representative of the value of the difference in tumor size. Thirty-six patients with adjuvant chemotherapy in 66 patients in the surgery group showed significant longer RFS than those without (24.1 months vs. 6.3 months, respectively;  $p=0.011$ ) but no difference was seen in comparison of OS (40.8 months vs. 49.5 months, respectively;  $p=0.994$ ). No significant difference of the number of patients with and without adjuvant chemotherapy was seen between synchronous and metachronous, unilateral and bilateral,  $N \geq 5$  and  $N<5$ , and  $R \geq 5$  and  $R<5$  ( $p=0.891, p=0.292, p=0.915, p=0.302$ , respectively), which indicated that adjuvant chemotherapy did not affect the comparison of RFS and OS in several predictive factors.

No significant difference was seen in age, gender, primary tumor location, and largest diameter of tumors between surgery and chemotherapy; however, a significant difference was observed

regarding time of occurrence, tumor distribution, the number of tumors, the difference in size of tumors and tumor markers between surgery and chemotherapy. In contrast to patients in the surgery group, patients in the chemotherapy group had several poor prognostic risk factors. To address the association between size of the liver tumor and its localization in patients' liver, we compared size of the liver tumor between right and left hepatic lobe in 31 patients with single liver metastasis. No significant difference of size of the liver tumor was seen between them ( $p=0.941$ ). In addition, no significant difference was seen between 4 segments of the liver, anterior segment of right hepatic lobe, posterior segment of right hepatic lobe, medial segment of left hepatic lobe and lateral segment of left hepatic lobe ( $p=0.862$ ).

Univariate analysis was performed to elucidate the impact of these factors on the outcome of RFS and OS in the surgery group (Table 2). This analysis revealed a significant difference in RFS concerning time of occurrence, the number of tumors present and the ratio of tumor diameters ( $p=0.015$ ,  $p=0.001$ ,  $p<0.001$ , respectively; Table 2). However, a significant difference in OS was observed only regarding the ratio of tumor diameters ( $p=0.001$ , Table 2). Comparison of survival curves for RFS and OS according to several factors such as time of occurrence, tumor distribution, the number of tumors and difference in tumor size are presented in (Figure 2). To independently verify prognostic factors affecting the outcome, we chose time of occurrence, the number of tumors present and the ratio of tumor diameters for multivariate analysis. In addition, tumor distribution was also included because it can be a prognostic factor that can be estimated in imaging studies before surgery as well as other parameters. Multivariate analysis revealed that the ratio of tumor size was the only independent prognostic factor regarding both RFS and OS (Table 3). To assess malignant potential, such as recurrence in the remnant liver, we focused on only liver metastasis after surgery, suggesting the liver recurrence occurred significant frequently in the patients with synchronous metastases and  $R \geq 5$ , but not in other risk factor patients (Table 4).

Next, we compared the impact of surgery with that of chemotherapy on outcome. To elucidate the significance of surgery, time to relapse regarding RFS in the surgery group was compared with time to progression regarding PFS in the chemotherapy group. There was a significant difference between them; the median RFS was 14.5 months in the surgery group versus a median PFS of 7.0 months in the chemotherapy group ( $p=0.001$ ). The surgery group achieved a significantly better OS than the chemotherapy group; the median OS was 46.3 months in the surgery group versus 22.1 months in the chemotherapy group ( $p=0.001$ ). Comparison of survival curves between RFS in the surgery group and PFS in the chemotherapy group is presented in Figure 3A. Comparison of the survival curves for OS between the surgery group and the chemotherapy group is presented in Figure 3B.

In consideration of the important prognostic risk factors such as time of recurrence, tumor distribution, the number of tumors and the ratio of tumor diameters, we compared the outcome between the surgery group and the chemotherapy group. Features involving the presence of synchronous tumors, bilateral distribution,  $N \geq 5$  and  $R \geq 5$  were associated with a poorer prognosis concerning RFS and PFS than those involving the presence of metachronous tumors, unilateral distribution,  $N<5$  and  $R<5$ . RFS in the surgery group and PFS in the chemotherapy group were compared in patients with these prognostic risk factors.

Median RFS in the surgery group and PFS in the chemotherapy group, and OS between the surgery group and the chemotherapy group

Variables	Median RFS and PFS (months)	p-value	Median OS (months)	p-value
Synchronous liver metastases				
Surgery (n=27)	RFS=12.2	0.144	46.7	0.013
Chemotherapy (n=13)	PFS=8.4		22.0	
Bilateral liver metastases				
Surgery (n=22)	RFS=14.5	0.035	49.1	0.003
Chemotherapy (n=12)	PFS=7.0		19.1	
Number of liver metastases ≥ 5				
Surgery (n=15)	RFS=9.9	0.699	42.0	0.017
Chemotherapy (n=11)	PFS=8.2		16.2	
Ratio of tumor diameter ≥ 5				
Surgery (n=8)	RFS=3.7	0.243	32.9	0.610
Chemotherapy (n=7)	PFS=5.8		10.5	
RFS: Relapse Free Survival; PFS: Progression Free Survival; OS: Overall Survival				

**Table 5:** Comparison of median relapse-free survival in the surgery group and progression-free survival in the chemotherapy group, and overall survival between the surgery group and the chemotherapy group in consideration of several prognostic risk factors.

were compared (Table 5) in consideration of several prognostic risk factors: bilateral tumors, number of liver metastases  $\geq 5$  ( $N \geq 5$ ) and  $R \geq 5$ . Patients with synchronous tumors in the surgery group achieved better RFS than PFS in the chemotherapy group, but the difference was not significant (12.2 versus 8.4 months, respectively;  $p=0.144$ ). Patients with bilateral tumors in the surgery group had a significantly better RFS than PFS in the chemotherapy group (14.5 and 7.0 months, respectively;  $p=0.035$ ). Patients with  $N \geq 5$  in the surgery group achieved a better RFS than PFS in the chemotherapy group, but the difference was not significant (9.9 and 8.2 months, respectively;  $p=0.699$ ). Patients with  $R \geq 5$  in the surgery group achieved a poorer RFS than PFS in the chemotherapy group, but the difference was not significant (3.7 and 5.8 months, respectively;  $p=0.243$ ). There was no significant difference between the surgery and chemotherapy groups regarding prognostic risk factors, with the exception of bilateral metastases. Comparisons of survival curves for the RFS in the surgery group and PFS for the chemotherapy group in consideration of several prognostic risk factors are presented in Figures 3C, 3E, 3G and 3I.

In comparing patients with these prognostic risk factors, those with synchronous metastases in the surgery group achieved a significantly better OS than those in the chemotherapy group (46.7 and 22.0 months, respectively;  $p=0.013$ ). Patients with bilateral metastases in the surgery group had a significantly better OS than those in the chemotherapy group (49.1 and 19.1 months, respectively;  $p=0.003$ ). In addition, patients with  $N \geq 5$  in the surgery group displayed significantly better OS than those in the chemotherapy group (42.0 and 16.2 months, respectively;  $p=0.017$ ). However, patients with  $R \geq 5$  did not exhibit a significant difference in OS between surgery and chemotherapy (32.9 and 10.5 months, respectively;  $p=0.610$ ). Comparisons of the survival curves for OS between the surgery group and the chemotherapy group in consideration of several prognostic risk factors are presented in Figures 3D, 3F, 3H and 3J.

**Discussion**

The present study demonstrated the effect of difference in tumor size assessed in pretherapeutic imaging on the identification of CRC patients with resectable liver metastases who required chemotherapy before surgery. No difference in outcome was found using surgery

and chemotherapy for those patients with  $R \geq 5$ . Chemotherapy could be an alternative to forward surgery to address oncological concerns regarding the tumor, such as latent metastases or poor treatment outcome. Otherwise surgery should proceed in patients regardless of tumor distribution and the number of tumors; this is because surgery was found to achieve a significantly better OS than chemotherapy, with the exception of patients with  $R \geq 5$ .

Previous studies have reported that bilateral metastases and  $N \geq 5$  metastases were prognostic risk factors that could be used only in studies involving preoperative imaging [14,17,20,25]. Conversely, other studies have found that these two parameters were not prognostic risk factors [4,19,22-24,27]. These factors are still contentious concerning the prediction of prognosis. Synchronous metastases have also been reported to have poorer prognosis than metachronous metastases [4,12]. In our study, patients with synchronous metastases and  $N \geq 5$  had significantly poorer prognosis in relation to RFS than those with metachronous metastases and  $N < 5$ . However, no difference in OS was observed in comparing patients with and without prognostic risk factors, according to distribution and the number of tumors. Indeed, these prognostic risk factors did not impact on OS in the surgery group.

The treatment strategy that surgery should be undertaken before chemotherapy is generally accepted for technically resectable CRC liver metastases in Japan. Advances in surgical techniques have led to changes in the criteria for resectability. The requirement for the remaining liver remnant to be equivalent to 30% of the original liver volume is considered to be the most critical factor [28]. Even the presence of disease outside of the liver no longer automatically excludes surgery provided that it is also resectable [29]. Indeed, there is no clear definition of what constitutes technically resectable liver tumors [30]. Technical considerations pertaining to the resectability of the tumors were defined using several criteria, but oncological concerns remain. Treatment of liver metastases should be assessed with respect to both technical and oncological viewpoints. Surgery should be used for the treatment of CRC liver metastases that can be resected technically and ontologically; otherwise, chemotherapy is an alternative.

In the current study, we proposed the utility of difference in tumor size in the prediction of malignant potential, including latent metastases and treatment outcome in pretherapeutic imaging studies. A mixture of small and large tumors may represent the possible existence of latent metastases, suggesting that the existence of small tumors facilitates the development of latent metastases. While no analysis has ever been conducted to explore the significance of difference in tumor size of liver metastasis in CRC patients, some papers showed two different features of tumor growth in other types of cancer, such as locally growth without metastasis and quickly development of metastasis regardless of growth speed [31,32]. The difference in tumor size may represent the feature of the later type of tumor growth, resulted in quick development of metastasis after surgery.

Intratumor heterogeneity may be involved in the biological behavior of liver metastasis [33], leading to the difference in tumor size. It has been shown that the expression of snail, a key marker for epithelia-mesenchymal transition (EMT), is useful for the identifying the heterogeneity of intra-tumor [34]. As Snail physically interacts with G9a [35], heterogeneous expression of Snail is very likely to be associated with the levels of G9a in tumors. Epigenetically, as G9a is the main writer of H3K9me2 [36] and it also plays a role in the maintenance of DNA methylation [37], higher levels of G9a might cause additional establishment of H3K9me2 and DNA methylation at tumor suppressor genes, such as p53 [38]. This makes G9a as a promising therapeutic

target for the treatment of malignant carcinoma. Actually, the levels of histone methyltransferase G9a are associated with malignancy of the liver tumor [39]. Therefore, understanding the heterogeneous expression of G9a in individual primary tumors might create a novel direction for assessment of the biological behavior of liver metastasis in patients.

Multivariate analysis revealed that  $R \geq 5$  was the only independent factor concerning the prediction of both RFS and OS. We then assessed the outcome of CRC patients with prognostic risk factors to compare surgery and chemotherapy. To elucidate the benefit of surgery in advance, time to relapse in patients with RFS in the surgery group was compared with time to progression in patients with PFS in the chemotherapy group. In patients with any prognostic risk factor, there was no significant difference between RFS and PFS, with the exception of patients with bilateral tumors. These patients are considered to have poor prognosis because most showed relapse shortly after surgery in clinical practice; thus, it is hard to determine whether surgery should be undertaken in these patients even if the tumor can be resected. In respect to OS, however, these patients with prognostic risk factors exhibited significantly better OS after surgery than after chemotherapy, with the exception of patients with  $R \geq 5$ . The deference in tumor size as a prognostic factor exhibited a different feature to other factors in that patients with  $R \geq 5$  had poor outcome regarding OS when treated with either surgery or chemotherapy. These results suggested that chemotherapy could be an alternative to forward surgery for patients with  $R \geq 5$  in addressing the oncological concerns regarding the tumor.

After surgery, the recurrence exclusively in the liver occurred significant frequently in the patients with synchronous metastases and  $R \geq 5$ ; this suggested that patients with synchronous tumors and  $R \geq 5$  probably had latent metastases in the remnant liver as well as a resected liver, rather than those with bilateral metastases and  $N \geq 5$ . Curative liver R0 resection for these patients with synchronous tumors and  $R \geq 5$  probably fails to remove latent metastases in the remnant liver, resulting in early relapse in the liver. In addition to the malignant potential of latent metastases, patients with  $R \geq 5$  treated with surgery had as poor an outcome concerning OS as those treated with chemotherapy.

## Conclusion

In conclusion, our study demonstrated the usefulness of difference in tumor size assessed in pre-therapeutic imaging in the selection of CRC patients with liver metastases who required chemotherapy before surgery. In CRC liver metastases, malignant potential such as the presence of latent metastases may differ between tumors with  $R < 5$  and  $R \geq 5$ . Because there is no difference in outcome using surgery or chemotherapy for those patients with  $R \geq 5$ , chemotherapy could be used as an alternative to address oncological concerns regarding the tumor. It is important, however, to interpret our results within the context of the study limitations; a further prospective study to assess the advantages of chemotherapy as the initial treatment is required to reach definitive conclusions in these patients.

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