Clinical Features and Prognostic Factors for Advanced Hepatocellular Carcinoma with Extrahepatic Metastasis

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Short running title: Hepatocellular Carcinoma with Extrahepatic Metastasis

Abstract

AIM: We evaluated whether differences in clinical features and survival exist between patients with advanced hepatocellular carcinoma (HCC) with extrahepatic metastasis who did and did not receive previous treatment.

METHODS: Between April 1998 and April 2012, 419 HCC patients (81 previously untreated patients and 338 previously treated patients) with extrahepatic metastasis were enrolled in this study. Differences in clinical features, including metastatic sites, were compared between the two groups. In addition, prognostic predictors among all patients and among the 81 previously untreated patients were analyzed.

RESULTS: The distribution of major metastatic sites was similar in both groups; the most frequent site of extrahepatic metastasis was the lungs, followed by the bones, lymph nodes, and adrenal glands. The median survival time among all 419 patients was 6.8 months. The 1-, 2-, 3-, and 5-year survival rates were 31.6%, 15.3%, 9.5%, and 2.3%, respectively. No significant differences in survival were observed between patients who did and did not receive previous treatment. Multivariate analysis revealed Child-Pugh classification, white blood cell count, neutrophil-lymphocyte ratio (NLR), and primary tumor stage to be independent predictors of survival for all patients and for the 81 previously untreated patients.

DISCUSSION: Differences in clinical features of patients with advanced HCC with extrahepatic metastasis were identified between patients who did and did not receive previous treatment.

Moreover, intrahepatic tumor status, Child-Pugh classification, white blood cell count, and NLR were demonstrated to be independent predictors of survival in HCC patients with extrahepatic metastasis.

Key words: hepatocellular carcinoma, extrahepatic metastasis, survival, previous treatment

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. Recent advances in imaging technology and implementation of surveillance programs for high-risk patients have led to increased detection of early-stage HCC, making curative therapies possible in some patients.[1, 2] However, the long-term survival of HCC patients remains unsatisfactory due to the high frequency of intra- and extrahepatic recurrence.[3, 4] In particular, the development of advanced HCC with extrahepatic metastasis hinders the use of curative therapies such as surgical resection or radiofrequency ablation, therefore contributing to poor survival. Prior to approval of sorafenib, many systemic chemotherapeutic regimens had been evaluated for patients with advanced HCC, but no effective therapeutic protocols have been identified.[5, 6] Recently, two randomized placebo-controlled trials demonstrated a survival benefit associated with sorafenib for advanced HCC patients, including those with extrahepatic metastasis.[7, 8] As a result, sorafenib has become the standard treatment for advanced HCC in the United States, Europe, Japan, and many other countries.[9, 10] However, even though sorafenib prolongs survival, this benefit remains unsatisfactory. Therefore, the development of new agents and/or combinations is necessary for patients with advanced HCC with extrahepatic metastasis. To identify optimal therapies, it is important to understand the clinical features and prognostic factors of these patients.

In our clinic, we have experienced many HCC patients whose disease progressed to extrahepatic metastasis despite administration of appropriate, repeated treatment for intrahepatic tumors, while we

also encounter patients who are initially diagnosed with HCC with extrahepatic metastasis at their first visit and therefore have not been previously treated for HCC. In patients with advanced HCC with extrahepatic metastasis, several previous studies only reported the clinicopathological features and prognosis of patients with HCC recurrence or a combined set of previously treated and untreated patients.[11-14] We hypothesized that previous treatment of intrahepatic tumors may affect the subsequent pattern of metastasis and prognosis among advanced HCC patients with extrahepatic metastasis. Therefore, it is important to clarify the characteristics of pure advanced HCC with extrahepatic metastasis that has not been previously treated. In addition, to our knowledge, no studies have compared the differences between patients with advanced HCC with extrahepatic metastasis who did and did not receive previous treatment. Consequently, in this study, we compared the clinical features and prognostic factors of patients with advanced HCC with extrahepatic metastasis who did and did not receive previous treatment.

Patients and Methods

Patients

Between April 1998 and April 2012, 419 patients were diagnosed with advanced HCC with extrahepatic metastasis at the Kurume University School of Medicine and were enrolled in this study. Hepatic functional reserve was determined using the Child-Pugh classification system. HCC tumor staging was performed using the 6th edition of the AJCC/UICC TNM classification system.[15] At diagnosis of HCC with extrahepatic metastasis, 338 patients (80.7%) had received previous treatment and 81 patients (19.3%) were untreated. Previous treatments for intrahepatic tumors were included hepatic resection in 65 patients (19.2%), percutaneous ethanol injection in 82 patients (24.3%), radiofrequency ablation in 83 patients (24.6%), transcatheter chemoembolization (TACE) in 204 patients (60.4%), and hepatic arterial infusion chemotherapy (HAIC) in 197 patients (58.3%). Patients included 353 males (84.3%) and 66 females, with a median age of 66.0 years (range, 15 to 92 years). Overall, 291 patients (69.5%) were positive for hepatitis C virus (HCV) infection and 75 patients (17.9%) were positive for hepatitis B virus (HBV) infection. A total of 208, 149, and 62 patients had Child-Pugh class A, B, and C, respectively, and 55, 186, and 175 patients had T0-2, T3, and T4 stage primary tumors, respectively. Extrahepatic metastases were observed in the lungs in 225 patients (53.7%), bones in 165 patients (39.4%), lymph nodes in 91 patients (21.7%), and adrenal glands in 44 patients (10.5%).

Diagnosis of HCC and Evaluation of Extrahepatic Lesions

Diagnosis of HCC was confirmed radiologically by hyperintensity in the arterial phase and washout in the venous-delay phase using either contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI),[9] and/or by elevated serum levels of alpha-fetoprotein (AFP) and des-gamma carboxy prothrombin (DCP). Tumor biopsy was performed in cases in which imaging findings were not consistent with characteristic features of HCC, or when tumor marker levels were not elevated. To evaluate extrahepatic metastasis, pulmonary lesions were detected on chest x-ray or chest CT, which were routinely performed at the first visit or every 3 to 6 months during the follow-up period. Additional examinations, such as bone scintigraphy and brain CT or MRI, were indicated when symptoms attributable to extrahepatic metastasis appeared. These examinations were also conducted when AFP and/or DCP levels were elevated and the elevation(s) could not be accounted for by the status of the intrahepatic lesion(s). Positron emission tomography/CT studies were performed as a supplemental examination.

Follow-up and Endpoint

After the diagnosis of HCC, each patient was followed carefully with respect to intrahepatic lesions and extrahepatic metastases. Serum biochemistries and AFP and DCP levels were measured, and ultrasonography was performed every 1 to 2 months. Contrast CT or MRI was performed every 2 to 6 months. Other imaging modalities were performed as necessary. The endpoint of this study was the

date of death, or last follow-up visit; the closing date was August 2012. The median duration of follow-up was 5.8 months (range, 0.2 to 111.9 months).

Statistical Analysis

Continuous variables are expressed as median values (range). Comparison analysis between patients who did and did not receive previous treatment was performed using the chi-square test for discrete variables and the Mann-Whitney U test for continuous variables. Overall survival was determined by Kaplan-Meier analysis, and differences between subgroups were compared with log-rank tests. A Cox proportional hazards stepwise model was used for univariate and multivariate analysis to identify any independent variables related to overall survival. Data from these models are expressed as hazard ratios (HR) and 95% confidence intervals (95% CI). All P-values were 2-tailed, and a level of < 0.05 was considered to be statistically significant. Statistical analysis was performed by SPSS software version 20 (SPSS Inc., Chicago, IL).

Results

Clinical Characteristics of Patients Who Did and Did Not Receive Previous Treatment

A comparison of the clinical characteristics of patients who did and did not receive previous treatment is shown in Table 1. Previously treated patients were significantly more likely to have HCV infection, Child-Pugh B+C, and a low primary tumor stage compared to previously untreated patients. Previously untreated patients had significantly lower neutrophil-lymphocyte ratio (NLR) and higher aspartate aminotransferase levels, white blood cell counts, platelet counts, and DCP levels compared to previously treated patients. Major metastatic sites were similar in both groups, which were the lungs, bones, lymph nodes, and adrenal glands.

Survival and Predictive Factors in All Patients

The cumulative survival curve of all 419 patients is shown in Figure 1. The median survival time (MST) for these patients was 6.8 months. The 1-, 2-, 3-, and 5-year survival rates were 31.6%, 15.3%, 9.5%, and 2.3%, respectively. Cox proportional hazards regression analysis was performed to identify independent predictors of survival (Table 2). The results of univariate analysis showed that Child-Pugh class (B+C), white blood cell count ($\geq 6.0\times 10^9$/L), NLR (≥ 4.0), AFP (≥ 200 ng/mL), and primary tumor stage (T4) were significant risk factors that adversely affected survival. Multivariate analysis identified Child-Pugh class (B+C; HR: 2.80, 95% CI: 2.23-3.52, $P < 0.001$), white blood cell count ($\geq 6.0\times 10^9$/L; HR: 1.82, 95% CI: 1.43-2.33, $P < 0.001$), NLR (≥ 4.0; HR:

1.89, 95%CI: 1.48-2.41, P < 0.001), AFP (≥ 200 ng/mL; HR: 1.48, 95%CI: 1.18-1.87, P = 0.001), and primary tumor stage (T4, HR: 1.43, 95% CI: 1.14-1.79, P = 0.002) were independent predictors of survival.

Survival and Predictive Factors in 81 Previously Untreated Patients

The cumulative survival curve of the 81 patients who did not receive previous treatment is shown in Figure 2. The MST for these patients was 7.4 months. The 1-, 2-, 3-, and 5-year survival rates were 33.7%, 18.8%, 11.9%, and 2.3%, respectively. No significant differences in survival between patients who did and did not receive previous treatment were observed. (P = 0.369) Cox proportional hazards regression analysis was performed to identify independent predictors of survival (Table 3). The results of univariate analysis showed that Child-Pugh class (B+C), white blood cell count (≥ 6.0× 10^9/L), NLR (≥ 4.0), and primary tumor stage (T4) were significant risk factors that adversely affected survival. Multivariate analysis identified Child-Pugh class (B+C; HR: 6.03, 95% CI: 3.31-10.99, P < 0.001), white blood cell count (≥ 6.0× 10^9/L; HR: 1.85, 95% CI: 1.09-3.15, P = 0.023), NLR (≥ 4.0; HR: 1.86, 95%CI: 1.01-3.43, P = 0.047), and primary tumor stage (T4, HR: 1.82, 95% CI: 1.10-2.99, P = 0.019) were independent predictors of survival.

Discussion

It is unclear whether previous treatment influences clinical features and prognosis in advanced HCC with extrahepatic metastasis. Therefore, we evaluated whether differences exist between patients with advanced HCC with extrahepatic metastasis who did and did not receive previous treatment. The present results show differences in various clinical features between these two groups of patients. Previously treated patients were more likely to have low white blood cell counts, low platelet counts, and poor liver function compared to previously untreated patients. One explanation for this observation is that leukocytopenia and thrombocytopenia may have been caused by previously administered anticancer drugs, because many patients had repeatedly undergone TACE and HAIC for intrahepatic tumors. In addition, repeated cancer recurrence and various treatments for intrahepatic tumors may have contributed to decreased liver function. Interestingly, previously untreated patients had more advanced-stage intrahepatic tumors compared to previously treated patients. Many studies have suggested that residual HCC following various treatments has increased malignant potential compared to untreated HCCs.[16-18] Therefore, we suggest that aggressive treatment results in earlier extrahepatic metastasis, despite a less advanced intrahepatic tumor stage, in previously treated patients. In this study, no significant differences in survival between the two groups were observed, although the groups differed in liver function and intrahepatic tumor stage. A possible explanation for this lack of difference is that worse liver function and an increased malignant potential of previously treated patients may offset the more advanced stage of intrahepatic

tumors of previously untreated patients.

Major sites of HCC metastasis include the lungs, lymph nodes, bones, and adrenal glands.[13, 14, 19-21] Yoo et al. reported that the most frequent metastatic sites in 251 previously untreated HCC patients with extrahepatic metastasis were the lungs (67.3%), lymph nodes (37.5%), bones (18.3%), and adrenal glands (7.6%).[20] Jun et al. indicated that in HCC patients with extrahepatic recurrence after hepatic resection, frequent metastatic sites included the lungs (41.9%), lymph nodes (19.9%), and bones (13.2%). In the present study, the most frequent site of extrahepatic metastasis was the lungs, followed by bones, lymph nodes, and adrenal glands, regardless of previous treatment of intrahepatic tumors. These results are similar to those of earlier reports, and suggest that previous treatment does not affect HCC metastatic patterns.

Several studies have reported that in HCC patients with extrahepatic metastasis, intrahepatic tumor status and Child-Pugh classification are independent prognostic factors.[12, 13, 20] Similarly, in the present study, multivariate analysis showed that these factors were independent predictors of survival in HCC patients with extrahepatic metastasis, regardless of previous treatment. Sorafenib is an oral systemic agent that extends overall survival and has become the standard treatment for advanced HCC patients, including those with extrahepatic metastasis. However, even the survival rates achieved with sorafenib remain unsatisfactory. Several studies have shown that features of primary tumor progression, such as vascular invasion, tumor size, and tumor number, are independent risk factors for extrahepatic metastasis following curative resection.[14, 22] Thus, control of

intrahepatic tumors may be important for the prevention of further extrahepatic metastasis. Pinter et al. reported that the MST of TACE alone (9.2 months) was similar to that of sorafenib alone (7.4 months) among patients with advanced HCC, including those with extrahepatic metastasis.[23] Jung et al. reported that in 240 HCC patients with extrahepatic metastasis, control of intrahepatic tumors was a favorable prognostic factor for survival: the MST of patients with treatment responses was significantly longer than that of patients who did not respond to treatment (521 vs. 170 days; $P < 0.001$).[14] Consequently, in advanced HCC patients with extrahepatic metastasis, a combination of intrahepatic local treatments and sorafenib may be useful, because the malignant potential of intrahepatic tumors is associated with extrahepatic spread and survival.

Hepatic reserve is important for hepatic resection and metabolism of anticancer drugs, including sorafenib. Pinter et al. showed that the risk of high-grade toxicities associated with sorafenib may be increased in patients with advanced liver dysfunction.[24] Our previous study of advanced HCC patients treated with HAIC showed that liver dysfunction necessitating treatment suspension or discontinuation more frequently occurred in patients with Child-Pugh class B disease than in patients with Child-Pugh class A disease.[25] Such insufficient treatment results in further liver dysfunction due to intrahepatic tumor progression, resulting in poor survival. Therefore, we presume that liver function is an important predictor of survival.

In this study, we also showed that an elevated white blood cell count involved with a high NLR was a significant independent predictor of survival in HCC patients with extrahepatic metastasis.

Recently, various markers of systemic inflammatory responses, including cytokines, C-reactive protein (CRP), and absolute blood neutrophil or lymphocyte count as well as their ratio (including NLR), have been investigated for their prognostic roles in cancer. Of these, NLR is one of the most simple and effective markers of inflammation and is linked with poor prognosis in various cancer types.[26-28] Several studies demonstrated that an elevated NLR was associated with worse survival in patients with HCC who underwent radiofrequency ablation, TACE, resection, and liver transplantation.[29-32] However, the exact relationship between a high NLR and poor prognosis remains unclear. One possibility is that patients with an elevated NLR have relative lymphocytopenia, leading to a weaker lymphocyte-mediated immune response to tumor due to a decreasing T4/T8 ratio.[33] As a result, these patients experience more tumor progression and therefore have a poor prognosis. Another possibility is that increased neutrophils modify and provide an adequate environment for tumor progression and development. Neutrophils have been shown to promote tumor growth and metastasis by secreting chemokines, vascular endothelial growth factor, and matrix metalloproteinase-9, which are involved in the angiogenesis that promotes tumor development.[34-36] Thus, a high neutrophil level offers a growth advantage for HCC through the increase of these pro-angiogenic factors, resulting in increased extrahepatic metastasis and worse survival in HCC patients.

In conclusion, we showed that differences in clinical features and no significant differences in survival exist between patients with advanced HCC with extrahepatic metastasis who did and did not

receive previous treatment. Moreover, we demonstrated that intrahepatic tumor status, Child-Pugh classification, white blood cell count, and NLR are independent predictors of survival in HCC patients with extrahepatic metastasis, regardless of previous treatment of intrahepatic tumors.

Figure legends

Figure 1. Cumulative survival curve for all 419 patients. The median survival time (MST) of all patients was 6.8 months. The 1-, 2-, 3-, and 5-year survival rates were 31.6%, 15.3%, 9.5%, and 2.3%, respectively.

Figure 2. Comparison of cumulative survival curves between patients who did and did not receive previous treatment. The median survival times (MST) of previously treated and untreated patients were 6.7 and 7.4 months, respectively (P = 0.369).

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Figure 1

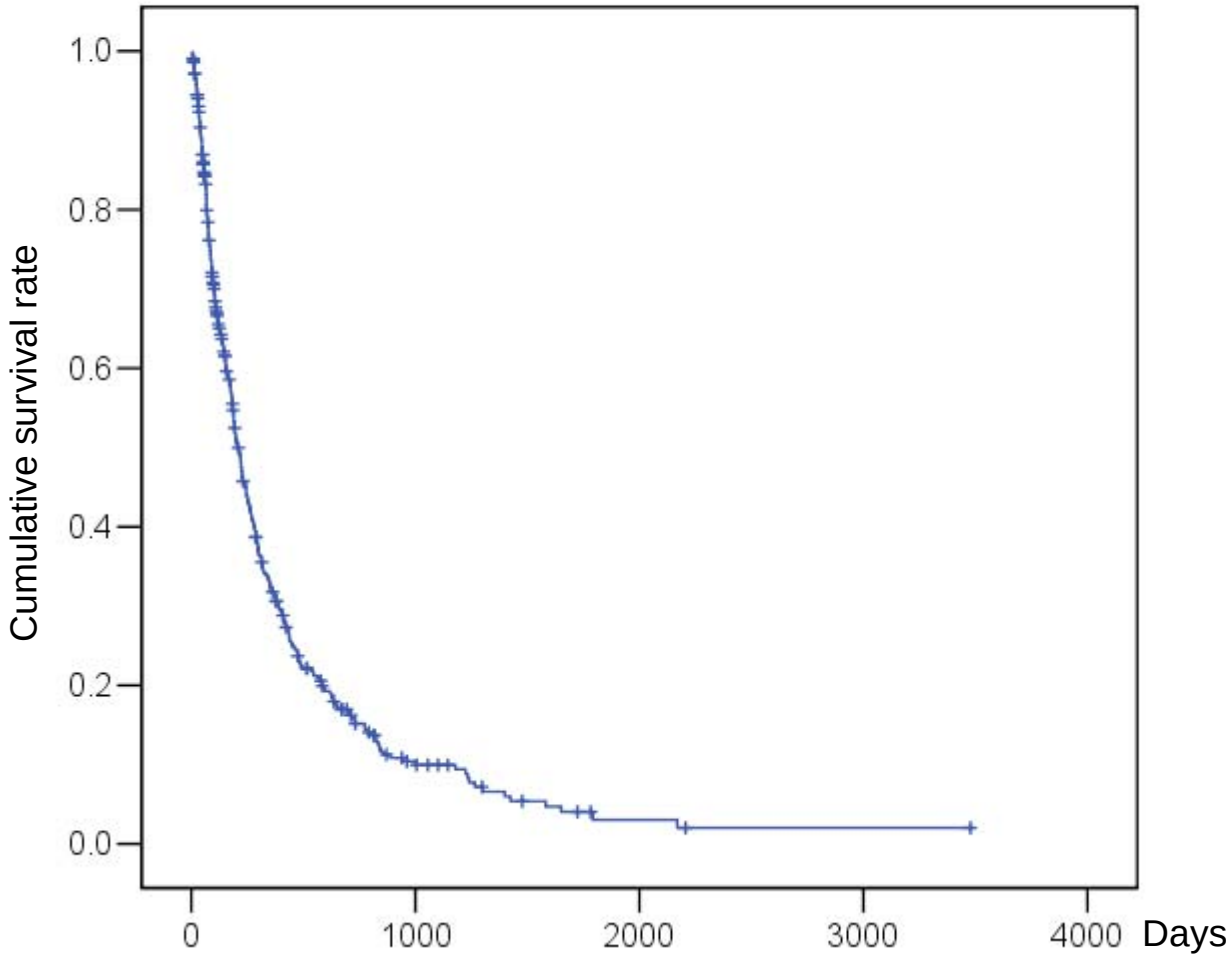
Cumulative survival rate
1.0
0.8
0.6
0.4
0.2
0.0
0
1000
2000
3000
4000
Days

Figure 2

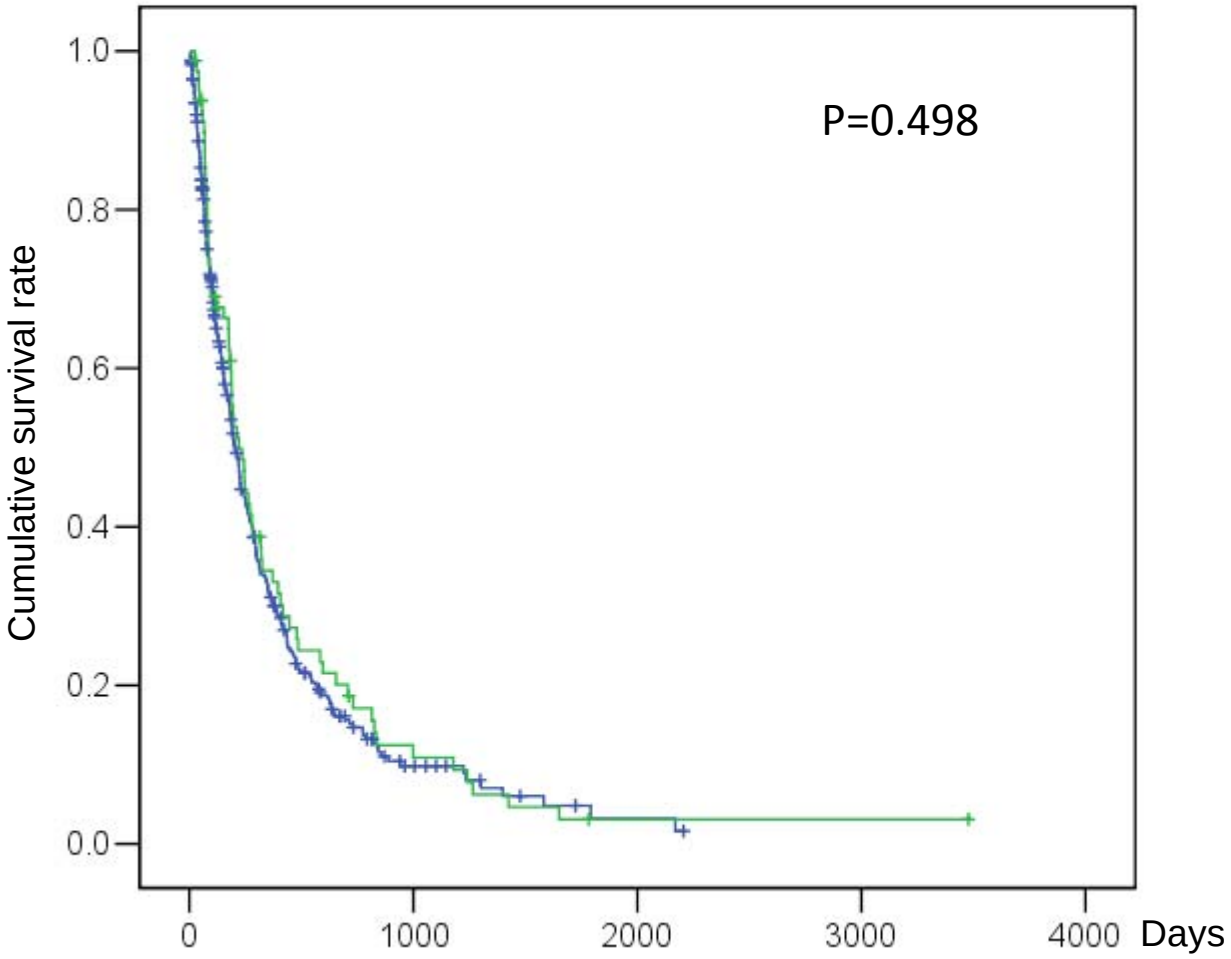
P=0.498
Cumulative survival rate
1.0
0.8
0.6
0.4
0.2
0.0
0
1000
2000
3000
4000
Days

Figure 3

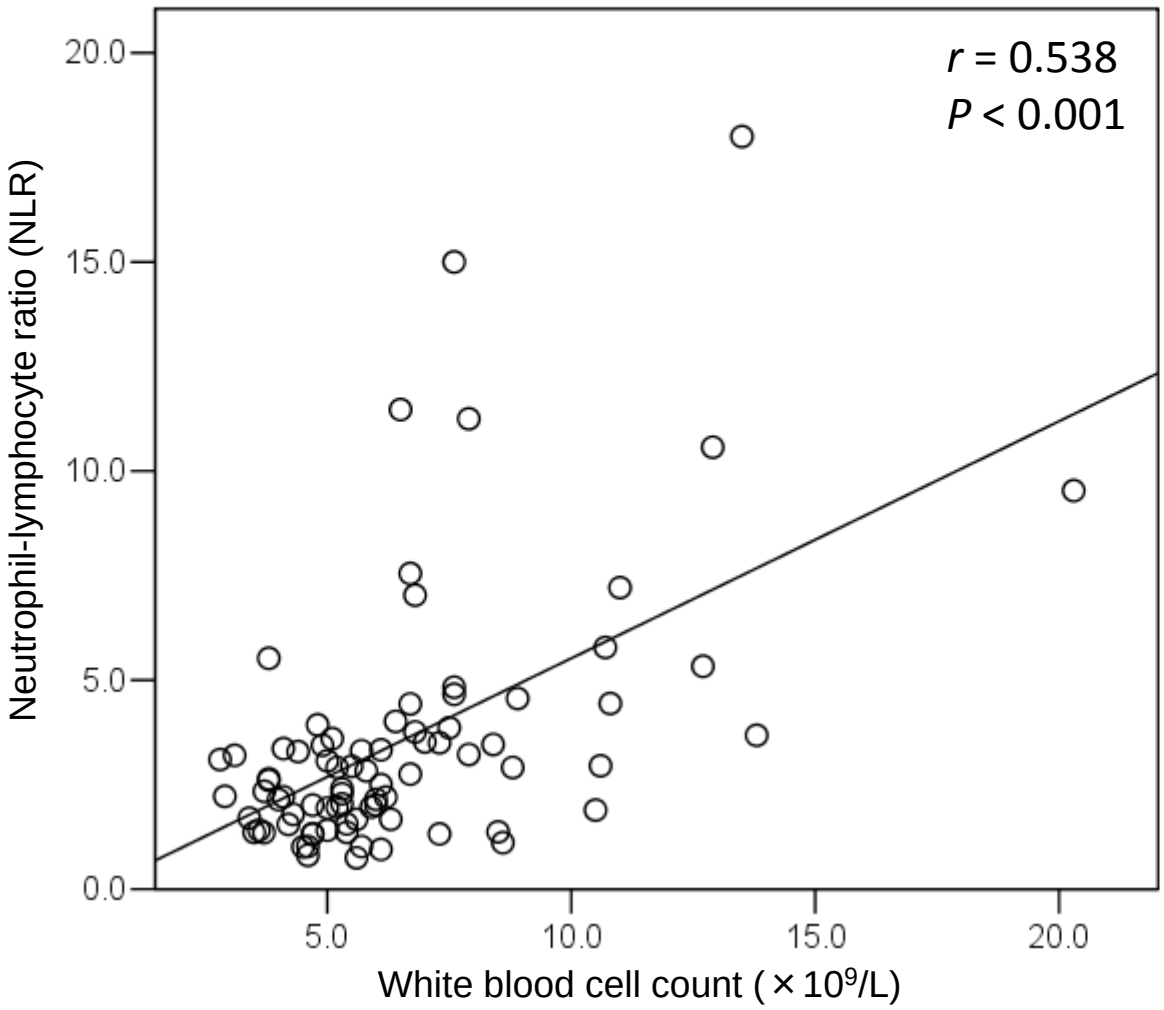
r = 0.538
P < 0.001
Neutrophil-lymphocyte ratio (NLR)
20.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
White blood cell count (×10⁹/L)

Table 1: Comparison of clinical characteristics between previously treated and untreated patients

	Previously untreated patients (n=81)	Previously treated patients (n=338)	*P* value
Gender (male/female)	70/11	283/55	0.523
Age (years)	63 (15-80)	67 (30-92)	<0.001
Etiology (HCV/HBV/Others)	45/18/19	246/57/40	0.006
Child-Pugh class (A/B/C)	56/17/8	152/132/54	<0.001
AST (U/L)	52 (12-280)	36 (6-412)	<0.001
White blood cell count ($\times 10^9$/L)	5.7 (2.8-20.3)	4.4 (1.4-20.5)	<0.001
NLR	2.8 (0.8-18.0)	3.1 (0.6-46.5)	0.038
Platelet count ($\times 10^9$/L)	139 (33-675)	101 (20-970)	<0.001
AFP (ng/mL)	1444.9 (2.2-3311794.0)	524.9 (1.5-1904794.0)	0.177
DCP (mAU/mL)	11400 (19-75000)	887.5 (8-75000)	<0.001
Primary tumor stage† (T0-2/T3/T4)	2/30/49	53/156/126	<0.001
Site of extrahepatic metastasis			
Lungs (present)	53.1% (43)	53.8% (182)	0.905
Bones (present)	43.2% (35)	38.5% (130)	0.410
Lymph nodes (present)	29.6% (24)	19.8% (67)	0.059
Adrenal glands (present)	13.6% (11)	9.8% (33)	0.306
Peritoneum and Pleura (present)	2.5% (2)	7.4% (25)	0.107
Diaphragm (present)	4.9% (4)	5.0% (17)	0.982
Brain (present)	1.2% (1)	3.0% (10)	0.387

Continuous variables presented as median (range)

HCV, hepatitis C virus; HBV, hepatitis B virus; AST, aspartate aminotransferase; NLR, neutrophil-lymphocyte ration; AFP, alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin

† TNM classification

Table 2: Univariate and multivariate analysis of survival in all 419 patients with extrahepatic metastasis

	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
Gender (male)	0.96 (0.71-1.29)	0.773		
Age (≥ 65 years)	1.10 (0.89-1.37)	0.374		
Etiology (HCV infection)	1.01 (0.80-1.28)	0.909		
Child-Pugh class (B+C)	2.82 (2.25-3.53)	<0.001	2.80 (2.23-3.52)	<0.001
AST (≥ 80 U/L)	1.15 (0.87-1.51)	0.335		
White blood cell count (≥ 6.0×10^9/L)	1.85 (1.45-2.35)	<0.001	1.82 (1.43-2.33)	<0.001
NLR (≥ 4.0)	2.48 (1.96-3.13)	<0.001	1.89 (1.48-2.41)	<0.001
Platelet count (≥ 120×10^9/L)	0.93 (0.75-1.15)	0.504		
AFP (≥ 200 ng/mL)	1.74 (1.39-2.18)	<0.001	1.48 (1.18-1.87)	0.001
DCP (≥ 200 mAU/ml)	1.10 (0.99-1.22)	0.073		
Primary tumor stage† (T4)	1.57 (1.27-1.95)	<0.001	1.43 (1.14-1.79)	0.002
Previous treatment (present)	1.13 (0.87-1.48)	0.370		
Site of extrahepatic metastasis				
Lungs (Present)	0.93 (0.75-1.15)	0.480		
Bones (Present)	1.23 (0.99-1.52)	0.066		
Lymph nodes (Present)	0.96 (0.74-1.24)	0.732		

HR, hazard ratio; 95% CI, 95% confidence interval; HCV, hepatitis C virus; AST, aspartate aminotransferase; NLR, neutrophil-lymphocyte ration; AFP, alpha-fetoprotein; DCP, des-gamma-carboxy Prothrombin

† TNM classification

Table 3: Univariate and multivariate analysis of survival in previously untreated patients with extrahepatic metastasis

	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
Gender (male)	1.33 (0.66-2.72)	0.427		
Age (≥ 65 years)	1.04 (0.64-1.68)	0.872		
Etiology (HCV infection)	0.79 (0.49-1.29)	0.351		
Child-Pugh class (B+C)	5.66 (3.22-9.94)	<0.001	6.03 (3.31-10.99)	<0.001
AST (≥ 80 U/L)	1.13 (0.66-1.93)	0.659		
White blood cell count (≥ 6.0×10^9/L)	1.97 (1.18-3.29)	0.009	1.85 (1.09-3.15)	0.023
NLR (≥ 4.0)	2.57 (1.44-4.56)	0.001	1.86 (1.01-3.43)	0.047
Platelet count (≥ 120×10^9/L)	1.25 (0.75-2.08)	0.387		
AFP (≥ 200 ng/mL)	1.43 (0.85-2.39)	0.175		
DCP (≥ 200 mAU/ml)	1.05 (0.56-1.96)	0.885		
Primary tumor stage† (T4)	1.64 (1.00-2.67)	0.048	1.82 (1.10-2.99)	0.019
Site of extrahepatic metastasis				
Lungs (Present)	0.93 (0.57-1.53)	0.783		
Bones (Present)	1.30 (0.800-2.13)	0.292		
Lymph nodes (Present)	1.23 (0.69-2.19)	0.482		

HR, hazard ratio; 95% CI, 95% confidence interval; HCV, hepatitis C virus; AST, aspartate aminotransferase; NLR, neutrophil-lymphocyte ration; AFP, alpha-fetoprotein; DCP, des-gamma-carboxy Prothrombin

† TNM classification