Am J Cancer Res 2024;14(6):2984-2993
www.ajcr.us /ISSN:2156-6976/ajcr0156197

Original Article

Heterogenous liver parenchymal enhancement in CT is a favorable prognosis of HCC after hepatic resection

Hsuan-Hwai Lin[1], Yi-Jen Peng[2], Chao-Feng Chang[1], Ping-Ying Chang[3], Ping-Chi Peng[1], Wei-Chou Chang[4], Teng-Wei Chen[5], Tsai-Yuan Hsieh[1]

[1]Division of Gastroenterology, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; [2]Division of Experimental Pathology, Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; [3]Division of Hematology/Oncology, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; [4]Department of Radiology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; [5]Division of General Surgery, Department of Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan

Received February 28, 2024; Accepted May 21, 2024; Epub June 15, 2024; Published June 30, 2024

Abstract: This study aimed to define the role of heterogeneity of liver parenchymal enhancement on computed tomography (CT) in the survival of patients with hepatocellular carcinoma (HCC) after hepatic resection. The medical records of patients with HCCs and who had undergone hepatic resection were retrospectively reviewed. The standard deviation (SD) of three different enhanced CT scan images was used to estimate the heterogeneity of liver parenchymal enhancement: SD of > 5.6, heterogenous enhancement, and SD of ≤ 5.6, homogeneous enhancement. A total of 57 patients had heterogenous enhancement, and 143 patients had homogeneous enhancement. The patients with heterogenous enhancement had longer disease-free and overall survivals than those with other enhancements (log-rank test, $P < 0.001$ and $P = 0.036$). The pathologic exam showed that heterogenous enhancement tended to develop septa in the peritumoral liver tissues. The prevalence of $CD8^+$ cells was significantly higher in the peritumor liver tissues with septa than in those without (0.83% vs. 0.26%, $P < 0.001$). The peritumoral CD8/Foxp3 ratio was higher in the liver tissues with septa than in those without (1.22 vs. 0.47, $P = 0.001$), and patients with CD8/Foxp3 of > 0.8 had better overall survival than those with CD8/Foxp3 of ≤ 0.8 (log-rank test, $P = 0.028$). In conclusion, patients who had undergone hepatic resection with a heterogenous liver parenchymal enhancement tended to develop hepatic septa, which was associated with a higher CD8/Foxp3 ratio and longer survival. Therefore, contrast-enhanced CT scans might be a useful tool to predict the outcome of HCC.

Keywords: CD8, Foxp3, hepatocellular carcinoma, heterogeneity of enhancement, prognosis

Introduction

Hepatocellular carcinoma (HCC) is the major type of primary liver cancer and is the fourth leading cause of cancer-related deaths worldwide [1]. Its most common risk factors are hepatitis B, hepatitis C, alcoholic liver disease, and nonalcoholic steatohepatitis [2, 3]. Patients with chronic hepatitis are diagnosed with HCC based on imaging or liver biopsy, and the choice of treatment depends on the cancer stage [4-6]. Hepatic resection is the preferred treatment option for patients with early-stage HCC [7]. Factors associated with the intrahepatic recurrence of HCC after resection include tumor size, histologic grade of HCC, microvascular invasion, and increased alpha-fetoprotein (AFP) level, respectively [8-12].

Heterogeneity of liver parenchyma on computed tomography (CT) scan is related to the liver cirrhosis severity [13], and the enhancement was significantly heterogeneous in patients with cirrhosis, in contrast that the non-cirrhotic liver presented with a homogeneous liver parenchyma [14]. Younger patients with HCC presented with better liver function, lesser severe liver cirrhosis, and more homogeneous liver parenchyma, but had more advanced cancer stage and higher AFP compared with older patients with HCC at diagnosis [15]. Therefore, the homogeneous liver parenchyma might be asso-

ciated with an advanced HCC stage in younger patients. Furthermore, an increase in liver parenchymal heterogeneity was associated with a low risk of liver metastases in patients with stage II/III colon cancer [16], suggesting that the tumor microenvironment of the heterogenous liver parenchyma might suppress the tumor growth and metastasis.

However, whether the heterogenous liver parenchyma prevented HCC from intrahepatic recurrence is still unclear. Therefore, this study aimed to define the role of liver parenchymal heterogeneity in the prognosis and survival of patients with HCC after hepatectomy.

Materials and methods

Study population

The medical records of patients with HCCs (Barcelona Clinic Liver Cancer [BCLC] stage A and B) and who had undergone hepatic resection as the initial HCC treatment modality were retrospectively reviewed at Tri-Service General Hospital between January 2015 and December 2018. Patients who underwent abdominal dynamic CT scan before resection were recruited.

Radiographic and clinical data

The protocol requirements for abdominal dynamic CT scan met the criteria recommended by the American Association for the Study of Liver Diseases guideline [17, 18]. The CT imaging from the institutional Picture Archiving and Communication Systems database was analyzed individually. The CT attenuation of the liver parenchyma was measured using manually drawn circular regions of interest (ROIs) measured at around 1 cm in diameter and placed with care to avoid the tumor, blood vessels, and areas of artifact (Supplementary Figure 1). Three ROIs were placed at the unenhanced and delayed phase images. The differenced enhancement of the liver parenchyma was measured (delayed phase of the liver - unenhanced liver) at the same location of ROIs. The heterogeneity of liver parenchymal enhancement was estimated using the standard deviation (SD) of the differenced enhancement of three ROIs [13]. The heterogeneity of liver parenchymal enhancement on CT has been associated with the liver fibrosis severity, and the mean SD of a normal liver was 5.6 [13]. Therefore, our patients were divided into two groups, with heterogeneous (SD > 5.6) and homogeneous (SD ≤ 5.6) liver parenchyma.

The clinical data and medical history of all patients were subsequently reviewed. The patient demographic data and previous hepatic resection laboratory examinations were recorded.

Immunohistochemical analysis

The formalin-fixed and paraffin-embedded liver tissue samples of the first quarter of reviewed patients who were included in this study were used for immunohistochemical analysis. Hematoxylin and eosin (H&E) stain was used to define the tumor and peritumoral liver tissues. Using the Beecher Instruments arraying device (Beecher Instruments, Silver Spring, MD), tissue cores with a 3-mm diameter were punched from the targeted tumor and peritumoral area of each tissue block to create the tissue array. Then, the consecutive sections were prepared for immunohistochemical analyses. Immunohistochemical stains were used to detect CD4, CD8, Foxp3, and CD11b antigens using the following antibodies: CD4 (Leica CD4-368-L-CE, diluted 1:100), CD8 (Leica CD8-4B11-L-CE, diluted 1:100), Foxp3 (GeneTex, GTX107737, diluted 1:100), and CD11b (Abcam, ab133357, diluted 1:100). To quantify the $CD4^+$, $CD8^+$, $Foxp3^+$, and $CD11b^+$ cells, ImageJ was used to count each tissue block (at least 10^7 cells in each tissue block). The proportion of positive cells among total cells was calculated.

Statistical analysis

All analyses were performed using SPSS statistics software, version 20 (IBM Co., Somers, NY, USA). The Chi-square or Fisher's exact tests were used to analyze categorical variables. Student's *t*-test was used to analyze continuous variables. The Kaplan-Meier method was used to identify the time-to-disease end-points; meanwhile, the log-rank test was used to estimate differences in survival between the two groups. A Cox proportional hazards model was used to compute multivariate hazard ratios (HRs) for the study parameters. All reported *P*-values were two-tailed, and the results were considered significant at $P < 0.05$.

Table 1. Clinical characteristics of study participants with a different parenchymal heterogeneity of liver

	SD > 5.6 n = 57	SD ≤ 5.6 n = 143	*p* value
Age, median (range)	63 (33-95)	62 (31-87)	0.61
Gender, male/female	36/21	112/31	0.027
Etiology of liver disease			0.56
HBV	35	92	
HCV	13	26	
HBV and HCV	0	5	
Alcohol	1	1	
Others	8	19	
Child-Pugh class			0.22
A	51	135	
B	6	8	
Bilirubin (mg/dL)	1.39 ± 2.63	0.83 ± 0.45	0.14
Albumin (gm/dL)	3.65 ± 0.73	3.75 ± 0.74	0.41
AST (U/L)	43.9 ± 25.1	42.1 ± 25.7	0.66
ALT (U/L)	42.4 ± 28.3	44.6 ± 39.0	0.70
Platelet count ($\times 10^3$/μl)	173.1 ± 82.6	168.2 ± 77.8	0.70
INR	1.23 ± 1.29	1.08 ± 0.75	0.31
AFP > 400 ng/ml (%)	12 (21.1)	23 (16.1)	0.40
ALBI grade			0.20
I	22	68	
II	28	67	
III	7	8	
FIB-4			0.80
< 1.45	12	28	
1.45-3.25	21	60	
> 3.25	24	55	
APRI			0.80
< 0.5	24	65	
0.5-1.5	26	58	
> 1.5	7	20	
Tumor size (cm)	4.7 ± 3.5	5.3 ± 3.7	0.32
BCLC stage			0.79
A	46	113	
B	11	30	
Microvascular invasion (%)	5 (8.8)	19 (13.3)	0.38
Histologic grade			0.77
Well differentiated	8	22	
Moderated differentiated	44	104	
Poorly differentiated	5	17	
Recurrence (%)	15 (26.3)	82 (57.3)	< 0.001

AFP: alpha-fetoprotein; ALBI grade: albumin-bilirubin grade; ALT: alanine aminotransferase; APRI: AST to platelet ratio index; AST: aspartate aminotransferase; BCLC: Barcelona-Clinic Liver Cancer; FIB-4: fibrosis-4; HBV: hepatitis B virus; HCV: hepatitis C virus; INR: international normalized ratio; SD: standard deviation. Results are expressed as mean ± SD.

Results

The clinical characteristics and prognostic factors were similar between patients with HCC with heterogenous and homogenous liver parenchyma

This retrospective study included a total of 200 patients. They had early- and intermediate-stage HCCs and underwent hepatic resection as the initial treatment modality for HCCs. Patients were divided into two groups based on the heterogeneity of liver parenchymal enhancement on CT, which was assessed using SD of the CT Hounsfield units (HU) of ROIs (Supplementary Table 1). There were 57 patients with an SD of > 5.6, suggesting a heterogeneous liver parenchyma, and 143 patients with an SD of ≤ 5.6, suggesting a homogeneous liver parenchyma. No significant differences were observed in patient demographics between the two groups, except for the gender ratio (**Table 1**). The proportion of male patients in the homogeneous group was higher than that in the heterogeneous group (78.3% vs. 63.2%, P = 0.027). The causes of liver disease were hepatitis B (63.5%), hepatitis C (19.5%), hepatitis B and C dual infection (2.5%), alcoholic hepatitis (1%), and others (13.5%), with hepatitis B being the majority cause of liver disease in both groups. Other basic clinical characteristics, such as aspartate aminotransferase, alanine transaminase, bilirubin, prothrombin time, and Child-Pugh class, were not significantly different between these two groups. The noninvasive assessment of the hepatic fibrosis, fibrosis-4 (FIB-4) index, revealed similar fibrotic stages in these two groups, and 79 (39.5%) patients had an index of > 3.25. Another noninvasive assessment of hepatic fibrosis, aspartate aminotransferase to platelet ratio

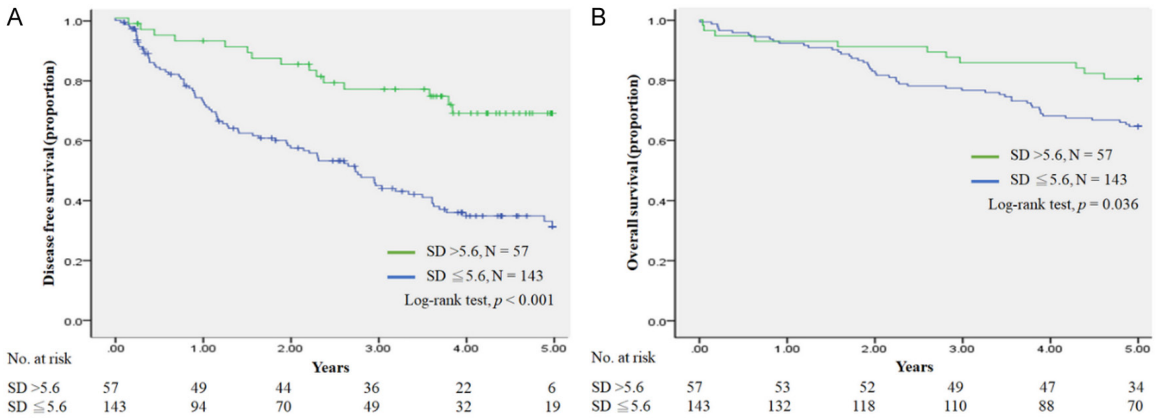


Figure 1. The heterogeneous liver parenchyma was associated with a lower risk of HCC recurrence after resection compared with the homogenous liver parenchyma. Comparison of (A) the disease-free survival (log-rank test $P < 0.001$) and (B) overall survival (log-rank test $P = 0.036$) curve based on the heterogeneity of liver parenchymal enhancement on CT scan. CT: computed tomography; SD: standard deviation.

index (APRI), also showed similar fibrotic status between these two groups, and 27 (13.5%) patients were > 1.5. Factors that may be associated with the HCC prognosis, such as tumor size, BCLC stage, AFP percentage of > 400 ng/ml, albumin-bilirubin (ALBI) grade, microvascular invasion, and histological grade of HCC, were not statistically significantly different between the two groups.

The heterogeneous liver parenchyma is associated with a lower risk of HCC recurrence after resection compared with the homogenous liver parenchyma

The clinical characteristics and prognostic factors were similar between patients with HCC with heterogenous and homogenous liver parenchyma; however, the HCC recurrent rate in the heterogeneous group was significantly lower than that in the homogenous group (26.3% vs. 57.3%, $P < 0.001$, **Table 1**). The Kaplan-Meier method was used to analyze disease-free survival and overall survival. The median disease-free survival of patients with SD of > 5.6 was not reached, and that of the patients with SD of ≤ 5.6 was 32.9 (range, 25.5-40.4) months. Patients with SD of > 5.6 had significantly longer disease-free survival than those with SD of ≤ 5.6 (log-rank test $P < 0.001$, **Figure 1A**). Furthermore, both the median overall survival of patients with SD of > 5.6 and SD of ≤ 5.6 was not reached. Patients with an SD of > 5.6 had significantly longer overall survival than those with an SD of ≤ 5.6 (log-rank test $P = 0.036$, **Figure 1B**).

A Cox proportional hazards model was used to adjust the factors that might be related to the HCC prognosis, such as gender, tumor size, Child-Pugh class, BCLC stage, AFP percentage of > 400 ng/ml, ALBI grade, FIB-4, APRI, microvascular invasion, and histologic grade of HCC, and the risk of recurrence in patients with HCC with SD of ≤ 5.6 was still significantly higher than those with SD of > 5.6 (HR 4.03, 95% confidence interval [CI]: 2.22-7.29, $P < 0.001$, **Table 2**). Patients with BCLC stage B also had a higher risk of HCC recurrence than patients with BCLC stage A (HR 2.48, 95% CI: 1.39-4.42, $P = 0.002$). Furthermore, patients with microvascular invasion also had a higher risk of HCC recurrence than those without (HR 1.95, 95% CI: 1.04-3.64, $P = 0.037$). No significant differences in the risk of recurrence were observed in terms of gender, FIB-4, APRI, tumor size, AFP percentage of > 400 ng/ml, and histologic grade of HCC ($P > 0.05$).

Pathological findings in different heterogeneities of the liver parenchyma

To investigate the mechanism associated with heterogenous liver parenchyma and favorable prognosis, the pathological findings in different heterogeneity of the liver parenchyma were examined. The 50 paraffin-embedded tissue blocks were stained with H&E, and 24 samples presented with septa in the peritumoral liver tissue (**Figure 2A**). Patients with heterogeneous liver parenchyma were significantly higher at developing septa compared with patients wi-

Table 2. Cox proportional hazards model for the HCC recurrence after resection

Risk factors	Hazard ratio (95% CI)	*p* value
Liver parenchymal enhancement		
SD > 5.6	1	
SD ≤ 5.6	4.03 (2.22-7.29)	< 0.001
Gender		
Female	1	
Male	0.95 (0.58-1.56)	0.84
Etiology		
Others	1	
HBV	1.10 (0.72-1.68)	0.84
Child-Pugh class		
A	1	
B	1.46 (0.62-3.45)	0.39
AFP		
≤ 400 ng/ml	1	
> 400 ng/ml	0.80 (0.44-1.47)	0.47
ALBI grade		
I	1	
II	1.39 (0.86-2.22)	0.18
III	0.78 (0.29-2.12)	0.63
FIB-4		
< 1.45	1	
1.45-3.25	1.36 (0.69-2.71)	0.38
> 3.25	1.11 (0.45-2.70)	0.63
APRI		
< 0.5	1	
0.5-1.5	1.40 (0.80-2.45)	0.24
> 1.5	2.74 (1.15-6.50)	0.023
Tumor size	1.01 (0.95-1.08)	0.69
BCLC stage		
A	1	
B	2.48 (1.39-4.42)	0.002
Microvascular invasion		
Without	1	
With	1.95 (1.04-3.64)	0.037
Histologic grade		
Well differentiated	1	
Moderated differentiated	1.61 (0.82-3.13)	0.16
Poorly differentiated	2.15 (0.91-5.11)	0.083

AFP: alpha-fetoprotein; ALBI grade: albumin-bilirubin grade; APRI: AST to platelet ratio index; BCLC: Barcelona-Clinic Liver Cancer; CI: confidence interval; FIB-4: fibrosis-4; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; SD: standard deviation.

th homogenous liver parenchyma (100% vs. 33.3%, P = 0.001, **Table 3**). The prevalence of intratumoral $CD4^+$, $CD8^+$, $Foxp3^+$, and $CD11b^+$ cells (**Figure 2B-E**) were not significantly different between patients with and without peritumoral septa (**Table 3**). However, the prevalence of $CD8^+$ cells was significantly higher in the peritumor liver tissues with septa than that in the peritumoral liver tissues without septa (0.83% vs. 0.26%, P < 0.001, **Table 3**). The prevalence of $Foxp3^+$ cells was also higher in the peritumor liver tissues with septa than in those without septa, but it did not reach statistical significance (1.05% vs. 0.59%, P = 0.076, **Table 3**). No significant difference in peritumoral $CD4^+$ and $CD11b^+$ cells was observed in peritumoral liver tissues with and without septa.

An increased intratumoral CD8/Foxp3 ratio is associated with a favorable prognosis of HCC after hepatic resection [19]. In our study, the intratumoral CD8/Foxp3 ratio in patients with peritumoral septa was higher than that in those without, but it did not reach statistical significance (0.62 vs. 0.49, P = 0.32, **Table 3**). The peritumoral CD8/Foxp3 ratio was significantly higher in the peritumor liver tissues with septa than in those without septa (1.22 vs. 0.47, P = 0.001, **Table 3**). The median CD8/Foxp3 ratio of early HCC pathologic stage T1 was 0.8 [19]. Using it as a cut-off value, 17 and 33 patients with HCC had the peritumoral CD8/Foxp3 ratio of > 0.8 and ≤ 0.8, respectively, in this study. Four patients with a peritumoral CD8/Foxp3 ratio of > 0.8 and 22 patients with a peritumoral CD8/Foxp3 ratio of ≤ 0.8 had recurrent HCCs within 2 years after resection (23.5% vs. 66.7%, P = 0.004). Furthermore, patients with a peritumoral CD8/Foxp3 ratio of > 0.8 had better disease-free survival after resection than patients with ≤ 0.8 (log-rank test P = 0.043, **Figure 3A**). The overall survival was significantly longer for patients with a peritumoral CD8/Foxp3 ratio of > 0.8 than those with a peritumoral CD8/Foxp3 ratio of ≤ 0.8 (log-rank test P = 0.028, **Figure 3B**).

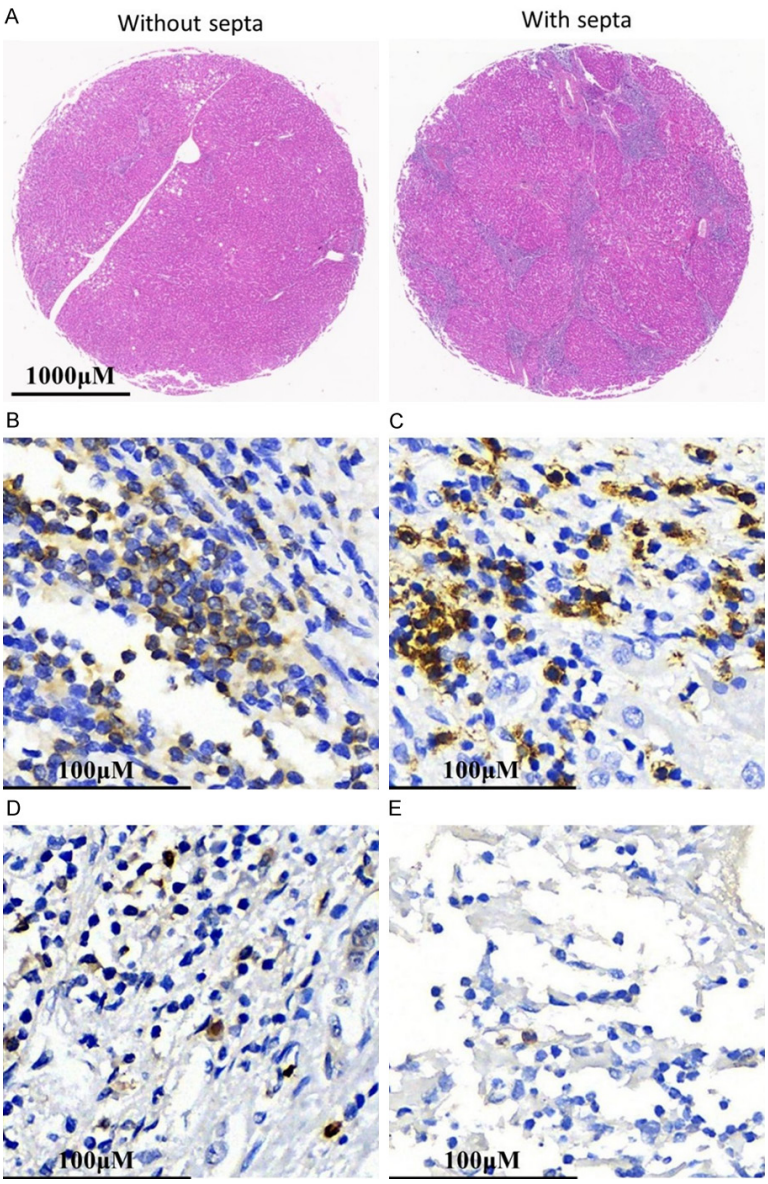


Figure 2. The H&E and immunohistochemical stains of HCC. (A) The H&E stain showing the peritumoral liver tissue sections without or with septa, and the immunohistochemical stain showing (B) $CD4^+$, (C) $CD8^+$, (D) $Foxp3^+$, and (E) $CD11b^+$ cells.

Discussion

This retrospective study reviewed the clinicopathologic data of patients with early- and intermediate-stage HCC who had undergone hepatic resection as the first treatment modality. Patients with HCC with heterogenous liver parenchymal enhancement on CT scan had a longer disease-free survival and overall survival than those with homogenous liver parenchymal enhancement. Furthermore, patients with heterogenous liver parenchyma tended to develop peritumoral septa, whereas those with peritumoral septa were likely to have a higher CD8/Foxp3 ratio, a lower risk of early recurrence, and a favorable overall survival (Supplementary Figure 2).

Liver cirrhosis is a well-known risk factor for HCC [20]; therefore, identifying liver cirrhosis is an important issue during the regular follow-up of patients with chronic hepatitis. A liver biopsy is considered the best method to evaluate the severity and diagnosis of liver fibrosis [21]. However, it might not be the gold standard. Liver biopsy has some limitations, such as interobserver variations and sampling variability [22, 23]. Additionally, the chronic liver disease would cause a bleeding tendency that increased the risk for post-biopsy hemorrhage. Alternatively, noninvasive methods for liver fibrosis, such as FIB-4 and APRI, could provide diagnostic ability [24, 25]. In this study, patients without significant difference in FIB-4 and APRI between SD of > 5.6 and SD of ≤ 5.6 groups, and most patients did not have advanced liver fibrosis, such as FIB-4 of > 3.25 or APRI of > 1.5, and 93% of patients had Child-Pugh class A (**Table 1**). Hepatic fractional extracellular space is another noninvasive method for liver fibrosis using CT scan [26], and no difference in the hepatic fractional extracellular space was observed between the two groups (Supplementary Table 1), suggesting a similar severity of liver fibrosis between the homogeneity and heterogeneity of liver parenchymal enhancement when patients with early HCC and could undergo hepatic resection. Although the heterogeneity of liver parenchymal enhancement is associated with the severity of

Table 3. Liver parenchymal heterogeneity and immunohistochemical variables of study participants with or without hepatic septa

	With septa n = 24	Without septa n = 26	*p* value
Liver parenchymal heterogeneity			0.001
SD > 5.6	8	0	
SD ≤ 5.6	16	26	
$CD4^+$ cells (%)			
Intratumor	0.063 ± 0.073	0.070 ± 0.069	0.74
Peritumor	0.240 ± 0.615	0.084 ± 0.110	0.21
$CD8^+$ cells (%)			
Intratumor	0.41 ± 0.46	0.37 ± 0.46	0.79
Peritumor	0.83 ± 0.72	0.26 ± 0.27	< 0.001
$Foxp3^+$ cells (%)			
Intratumor	0.76 ± 0.77	0.76 ± 0.68	0.99
Peritumor	1.05 ± 1.19	0.59 ± 0.49	0.076
$CD11b^+$ cells (%)			
Intratumor	0.032 ± 0.046	0.023 ± 0.041	0.46
Peritumor	0.239 ± 0.906	0.037 ± 0.111	0.26
$CD8^+/Foxp3^+$			
Intratumor	0.62 ± 0.48	0.49 ± 0.41	0.32
Peritumor	1.22 ± 0.96	0.47 ± 0.32	0.001

HCV: hepatitis C virus; INR: international normalized ratio; SD: standard deviation. Results are expressed as mean ± SD.

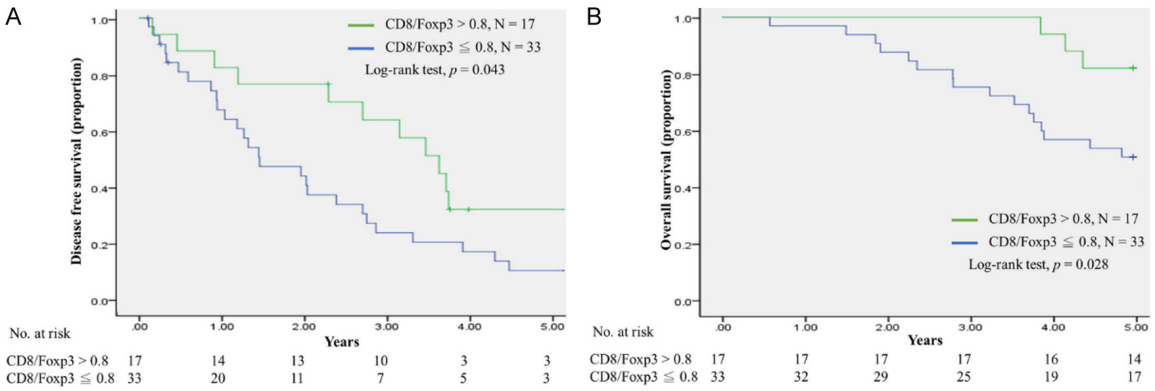


Figure 3. The disease-free and overall survival was significantly longer for patients with a peritumoral CD8/Foxp3 ratio of > 0.8 than those with a peritumoral CD8/Foxp3 ratio of ≤ 0.8. Comparison of (A) the disease-free survival (log-rank test *P* = 0.043) and (B) overall survival (log-rank test *P* = 0.028) curve based on the peritumoral CD8/Foxp3 ratio.

liver fibrosis [13], the majority of patients recruited in this study did not have advanced liver fibrosis; thus, showing the difference of fibrosis in this patient population might be difficult with this method. In our study, all patients with HCC with heterogeneous liver parenchyma on CT scan had peritumoral septa. In contrast, only 38.1% of patients with homogeneous liver parenchyma had peritumoral septa (see **Table 3**). The septa in non-tumor hepatic tissue might hamper the distribution of the contrast medium evenly and then cause the heterogenous liver parenchyma on the CT scan after administering the contrast medium.

In our study, the heterogenous liver parenchyma is associated with the generation of septa in non-tumor tissues. Fibrotic septa are a unique pathological feature in the development of liver fibrosis [27]. In an animal study of liver

fibrosis, the $CD8^+$ T-cell administration could induce the expression of transforming growth factor β1 and collagen I and increase the area of liver fibrosis [28]. Furthermore, patients with liver cirrhosis had a higher proportion of $CD8^+$ T cells in the liver tissues compared with patients without cirrhosis [29]. These results suggested that $CD8^+$ T cells might play a profibrotic role in liver fibrosis [30]. In this study, the prevalence of $CD8^+$ cells was significantly higher in the peritumoral liver tissue with septa than that in the peritumoral liver tissue without septa (**Table 3**). Our finding is consistent with that of previous reports that $CD8^+$ T-cell is associated with liver fibrosis.

$CD8^+$ T cells have an anticancer effect in the tumor microenvironment of HCC [31]. However, its activity is negatively regulated by regulatory T cells (Tregs) that impaired the $CD8^+$ T-cell function and promoted HCC disease progression [32]. Patients with HCC with low intratumoral Tregs and high intratumoral $CD8^+$ T cells have longer survival and lesser recurrence compared with others after hepatic resection [33]. Furthermore, increased intratumoral CD8/Foxp3 ratio is related to a favorable HCC prognosis after hepatic resection [19]. However, the intratumoral CD8/Foxp3 ratio was not significantly different between the liver tissues with and without septa in this study. Interestingly, the CD8/Foxp3 ratio of peritumoral liver tissue was significantly higher in patients with HCC with septa than that without septa, and a higher CD8/Foxp3 ratio of the peritumoral liver tissue is associated with a lower risk of early recurrence and longer survival. These findings suggested that the heterogeneous liver parenchyma on contrast-enhanced CT scan is associated with the septal generation and increased CD8/Foxp3 ratio of the peritumoral liver tissue and then resulted in a better prognosis of HCC after hepatic resection. Therefore, the heterogeneous liver parenchyma might represent a favorable tumor microenvironment for cancer treatment.

In routine clinical practice, obtaining data on the HCC tumor microenvironment without hepatic resection is difficult. In this study, we provided an accessible and noninvasive method to obtain the phenotype of the HCC tumor microenvironment. It might be a useful method to guide adjuvant therapy after hepatic resection or curative radiofrequency ablation. For example, the heterogeneous liver parenchyma might present with an immunoactive tumor microenvironment that enhances the therapeutic effect of immunotherapy. To verify this assumption, further clinical trials are warranted.

Conclusions

According to this study, patients with HCC with heterogenous liver parenchymal enhancement on CT scan might develop fibrotic septa in peritumoral liver tissue, which is associated with a higher CD8/Foxp3 ratio and then results in a lower risk of early recurrence and a longer overall survival.

Acknowledgements

This paper is supported by the National Science and Technology Council of Taiwan (110-2314-B-016-039), Tri-Service General Hospital (TSGH-D-110043, TSGH-D-111082, and TSGHD-112084), and Medical Affairs Bureau Ministry of National Defense (MND-MAB-110-112).

Disclosure of conflict of interest

None.

Address correspondence to: Hsuan-Hwai Lin, Division of Gastroenterology, Tri-Service General Hospital, No. 325, Section 2, Cheng-Gong Road, Neihu District, Taipei 114, Taiwan. Tel: +886-287927409; ORCID: 0000-0003-0171-9044; Fax: +886-28792-7139; E-mail: redstone120@gmail.com

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.

[2] Yang JD and Roberts LR. Epidemiology and management of hepatocellular carcinoma. Infect Dis Clin North Am 2010; 24: 899-919, viii.

[3] Huang MY, Chung CH, Chang WK, Lin CS, Chen KW, Hsieh TY, Chien WC and Lin HH. The role of thiazolidinediones in hepatocellular carcinoma risk reduction: a population-based cohort study in Taiwan. Am J Cancer Res 2017; 7: 1606-1616.

[4] Bruix J, Reig M and Sherman M. Evidence-based diagnosis, staging, and treatment of pa-

tients with hepatocellular carcinoma. Gastroenterology 2016; 150: 835-853.
[5] Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR and Heimbach JK. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. Hepatology 2018; 68: 723-750.
[6] Ayuso C, Rimola J, Vilana R, Burrel M, Darnell A, Garcia-Criado A, Bianchi L, Belmonte E, Caparroz C, Barrufet M, Bruix J and Bru C. Diagnosis and staging of hepatocellular carcinoma (HCC): current guidelines. Eur J Radiol 2018; 101: 72-81.
[7] Reig M, Forner A, Rimola J, Ferrer-Fabrega J, Burrel M, Garcia-Criado A, Kelley RK, Galle PR, Mazzaferro V, Salem R, Sangro B, Singal AG, Vogel A, Fuster J, Ayuso C and Bruix J. BCLC strategy for prognosis prediction and treatment recommendation: the 2022 update. J Hepatol 2022; 76: 681-693.
[8] Shah SA, Cleary SP, Wei AC, Yang I, Taylor BR, Hemming AW, Langer B, Grant DR, Greig PD and Gallinger S. Recurrence after liver resection for hepatocellular carcinoma: risk factors, treatment, and outcomes. Surgery 2007; 141: 330-339.
[9] Hong G, Suh KS, Suh SW, Yoo T, Kim H, Park MS, Choi Y, Paeng JC, Yi NJ and Lee KW. Alpha-fetoprotein and (18)F-FDG positron emission tomography predict tumor recurrence better than Milan criteria in living donor liver transplantation. J Hepatol 2016; 64: 852-859.
[10] Lin HH, Peng YJ, Tsai MJ, Wu YY, Tsai TN, Huang HH, Shih YL, Chang WK and Hsieh TY. Upregulation of amphiregulin by retinoic acid and Wnt signalling promotes liver cancer cell proliferation. J Cell Physiol 2020; 235: 1689-1699.
[11] Marrero JA, Fontana RJ, Barrat A, Askari F, Conjeevaram HS, Su GL and Lok AS. Prognosis of hepatocellular carcinoma: comparison of 7 staging systems in an American cohort. Hepatology 2005; 41: 707-716.
[12] Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S and Makuuchi M. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. J Hepatol 2003; 38: 200-207.
[13] Taura T, Nakamura K, Takashima S, Kaminou T, Yamada R, Shuto T and Wakasa K. Heterogeneity of hepatic parenchymal enhancement on computed tomography during arterial portography: quantitative analysis of correlation with severity of hepatic fibrosis. Hepatol Res 2001; 20: 182-192.
[14] Tamada T, Ito K, Higaki A, Yoshida K, Kanki A, Sato T, Higashi H and Sone T. Gd-EOB-DTPA-enhanced MR imaging: evaluation of hepatic enhancement effects in normal and cirrhotic livers. Eur J Radiol 2011; 80: e311-316.
[15] Chang PE, Ong WC, Lui HF and Tan CK. Is the prognosis of young patients with hepatocellular carcinoma poorer than the prognosis of older patients? A comparative analysis of clinical characteristics, prognostic features, and survival outcome. J Gastroenterol 2008; 43: 881-888.
[16] Creasy JM, Cunanan KM, Chakraborty J, McAuliffe JC, Chou J, Gonen M, Kingham VS, Weiser MR, Balachandran VP, Drebin JA, Kingham TP, Jarnagin WR, D'Angelica MI, Do RKG and Simpson AL. Differences in liver parenchyma are measurable with CT radiomics at initial colon resection in patients that develop hepatic metastases from stage II/III colon cancer. Ann Surg Oncol 2021; 28: 1982-1989.
[17] Han S, Hu C, Moser CD, Fang Y, Chaiteerakij R, Shire AM, Miamen A, Yang D, Thorgeirsson S, Fernandez-Zapico ME and Roberts LR. Expression of SULF2 by HCC cells promotes tumorigenesis by inducing activation, proliferation and migration of hepatic stellate cells in the tumor Microenvironment. Gastroenterology 2013; 144 Suppl 1: S-941.
[18] Bruix J and Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology 2011; 53: 1020-1022.
[19] Mathai AM, Kapadia MJ, Alexander J, Kernochan LE, Swanson PE and Yeh MM. Role of Foxp3-positive tumor-infiltrating lymphocytes in the histologic features and clinical outcomes of hepatocellular carcinoma. Am J Surg Pathol 2012; 36: 980-986.
[20] Fattovich G, Stroffolini T, Zagni I and Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 2004; 127 Suppl 1: S35-S50.
[21] Bravo AA, Sheth SG and Chopra S. Liver biopsy. N Engl J Med 2001; 344: 495-500.
[22] Bedossa P and Carrat F. Liver biopsy: the best, not the gold standard. J Hepatol 2009; 50: 1-3.
[23] Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F and Poynard T; LIDO Study Group. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology 2005; 128: 1898-1906.
[24] Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS and Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003; 38: 518-526.

[25] Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D and Nelson M; APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006; 43: 1317-1325.

[26] Zissen MH, Wang ZJ, Yee J, Aslam R, Monto A and Yeh BM. Contrast-enhanced CT quantification of the hepatic fractional extracellular space: correlation with diffuse liver disease severity. AJR Am J Roentgenol 2013; 201: 1204-1210.

[27] Hernandez-Gea V and Friedman SL. Pathogenesis of liver fibrosis. Annu Rev Pathol 2011; 6: 425-456.

[28] Safadi R, Ohta M, Alvarez CE, Fiel MI, Bansal M, Mehal WZ and Friedman SL. Immune stimulation of hepatic fibrogenesis by CD8 cells and attenuation by transgenic interleukin-10 from hepatocytes. Gastroenterology 2004; 127: 870-882.

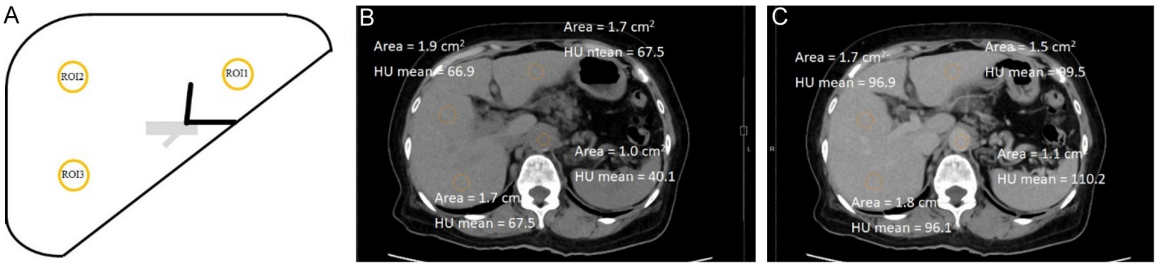
[29] Lebosse F, Gudd C, Tunc E, Singanayagam A, Nathwani R, Triantafyllou E, Pop O, Kumar N, Mukherjee S, Hou TZ, Quaglia A, Zoulim F, Wendon J, Dhar A, Thursz M, Antoniades CG and Khamri W. CD8(+)T cells from patients with cirrhosis display a phenotype that may contribute to cirrhosis-associated immune dysfunction. EBioMedicine 2019; 49: 258-268.

[30] Muhanna N, Horani A, Doron S and Safadi R. Lymphocyte-hepatic stellate cell proximity suggests a direct interaction. Clin Exp Immunol 2007; 148: 338-347.

[31] Wada Y, Nakashima O, Kutami R, Yamamoto O and Kojiro M. Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. Hepatology 1998; 27: 407-414.

[32] Fu J, Xu D, Liu Z, Shi M, Zhao P, Fu B, Zhang Z, Yang H, Zhang H, Zhou C, Yao J, Jin L, Wang H, Yang Y, Fu YX and Wang FS. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. Gastroenterology 2007; 132: 2328-2339.

[33] Gao Q, Qiu SJ, Fan J, Zhou J, Wang XY, Xiao YS, Xu Y, Li YW and Tang ZY. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. J Clin Oncol 2007; 25: 2586-2593.

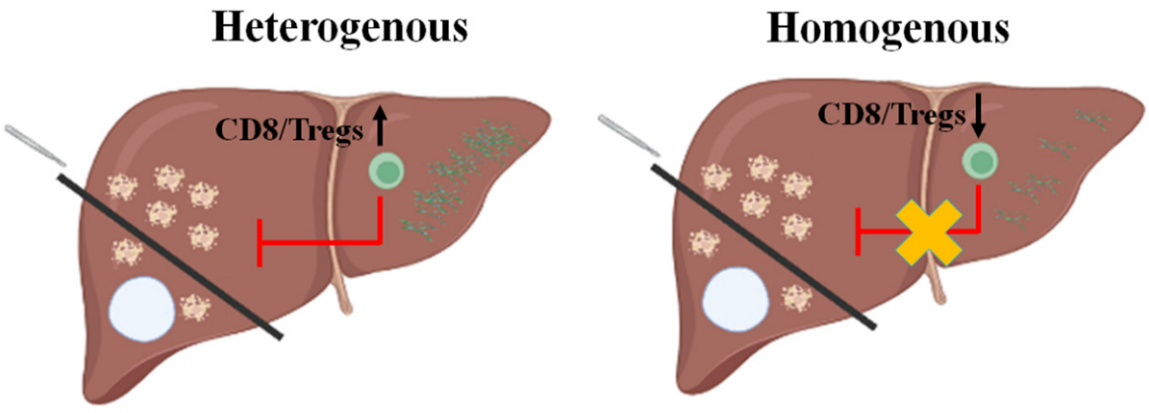


Supplementary Figure 1. Three ROIs were set as the schema (A) to measure the CT HU values at the unenhanced (B) and delayed phase images (C). CT, computed tomography; HU, Hounsfield units; ROI, regions of interest.

Supplementary Table 1. The CT imaging features of patients with a different parenchymal heterogeneity of liver

	SD > 5.6 n = 57	SD ≤ 5.6 n = 143	*p* value
Unenhanced scan (HU)			
ROI1	51.8 ± 9.0	55.4 ± 8.4	0.010
ROI2	55.4 ± 9.8	54.6 ± 9.3	0.57
ROI3	53.4 ± 11.3	53.9 ± 9.8	0.73
Delayed phase (HU)			
ROI1	96.8 ± 11.1	95.6 ± 11.8	0.50
ROI2	97.7 ± 14.3	95.3 ± 13.4	0.26
ROI3	90.8 ± 19.1	93.2 ± 13.0	0.30
Hepatic fractional extracellular space (%)[a]	35.1 ± 11.2	36.4 ± 11.5	0.47

CT: computerized tomography; HU: Hounsfield unit; ROI: regions of interest; SD: standard deviation. Results are expressed as mean ± SD. a: (delayed phase liver - unenhanced liver) × (100 - hematocrit)/(delayed phase aorta - unenhanced aorta).



Supplementary Figure 2. Schematic diagram depicts the heterogenous liver parenchyma tended to develop peritumoral septa, whereas those with peritumoral septa were likely to have a higher CD8/Tregs ratio, a suppression of free cancer cells, and a decrease in the recurrent rate. Tregs, regulatory T cells. Created with BioRender.com.