

## Relationship between Multicentric Occurrence of Hepatocellular Carcinoma and Histology of Noncancerous Hepatic Tissue in Patients with Chronic Hepatitis C

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The relationship between multicentric occurrence of hepatocellular carcinoma (HCC) and the histology of noncancerous hepatic tissue was investigated in 252 patients infected with hepatitis C virus (HCV) and surgically treated for HCC. One type of multicentric HCC had at least one tumor consisting of well-differentiated HCC, together with moderately or poorly differentiated HCC located in a separate region. The other type had an area of well-differentiated component around HCC with less differentiation in all occurrences. Noncancerous hepatic tissues were assessed using a histologic activity index score. Serum alanine aminotransferase (ALT) activity, the concentration of type 4 collagen, the grading score (severity of active hepatitis), and the staging score (degree of fibrosis) were significantly higher in patients with multicentric HCCs than in those without them. Platelet count was significantly lower in patients with multicentric HCCs. The prevalence of multicentric HCCs increased as the grading score and staging score increased. On univariate analysis, a low platelet count and high grading and staging scores were risk factors for multicentric HCCs. A high ALT activity and a high concentration of type 4 collagen tended to be risk factors. On multivariate analysis, high grading score and high staging score were independent risk factors. These findings indicate that active hepatitis and extensive fibrosis are responsible for the development of multicentric HCCs. Measurement of platelet count, ALT activity, and the concentration of type 4 collagen, and histologic assessment of noncancerous hepatic tissue provide information useful for estimation of the potential for multicentric carcinogenesis.

Key words: Hepatocellular carcinoma — Multicentric carcinogenesis — Hepatitis C virus — Active hepatitis — Cirrhosis

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are important factors in the development of hepatocellular carcinoma (HCC), and the mechanism of this development appears to differ between patients infected with HBV and HCV.<sup>1)</sup> In patients infected with HCV, chronic inflammation, liver cell necrosis and regeneration, and extensive fibrosis are responsible for the development of HCC, although the mechanism of hepatocarcinogenesis in these patients is still uncertain. These changes occur diffusely in the liver, and precancerous nodular lesions including adenomatous hyperplasia are often found throughout the liver. Most HCCs develop in steps through such precancerous lesions,<sup>2-5)</sup> and multicentric (multifocal) carcinogenesis is characteristic of HCC, especially in patients infected with HCV; the prevalence of multicentric carcinogenesis is higher in patients infected with HCV than in patients infected with HBV.<sup>6-11)</sup> During surgery, it is not uncommon for new nodules not found during preoperative examination to be detected.<sup>12)</sup> It has also been reported that continuous active hepatitis is a risk factor for recurrence after

resection of primary HCC, and that recurrent tumors include tumors of multicentric origin,<sup>13, 14)</sup> especially in patients infected with HCV.<sup>15-18)</sup> Therefore, it is important to determine the relationship between the occurrence of multicentric HCC and the histology of noncancerous hepatic tissue. However, this relationship has not been clarified in detail. In this study, we retrospectively investigated the relationship between multicentric occurrence of HCC and the histology of noncancerous hepatic tissue in patients with HCV-related HCC.

### PATIENTS AND METHODS

**Patients** The subjects were 252 patients who had undergone liver resection for HCV-related HCC at the Second Department of Surgery, Osaka City University Medical School between April 1991 and October 1998. Sera from all 252 patients were positive for HCV antibody (examined by second- or third-generation enzyme-linked immunosorbent assay; Ortho Diagnostic Systems, Tokyo). Sera from all patients were negative for hepatitis B surface antigen (examined by an enzyme immunoassay; International Reagents Corp., Kobe). Before surgery, patients underwent

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sonography, computed tomography (CT), angiography and (for most patients) magnetic resonance imaging studies. Since May 1993, CT during arteriography and CT during arteriportography were performed, if possible. Intraoperative sonography was also performed for patients, and when a nodule(s) not detected earlier was found, it was biopsied.

This study was conducted in accordance with the Helsinki Declaration and the guidelines of the Ethics Committee of our institution. Informed consent was obtained from each patient.

**Pathologic examination** Resected specimens were cut into serial slices 5 mm thick and fixed in 10% formalin. Biopsy specimens were also fixed in 10% formalin. Sections from trimmed paraffin blocks were stained with hematoxylin and eosin. The pathologic diagnosis was made based on the General Rules for the Clinical and Pathological Study of Primary Liver Cancer in Japan,<sup>19)</sup> with some modifications.<sup>20, 21)</sup> Well-differentiated HCC had high cell density, a high nucleus/cytoplasm ratio, strong cytoplasmic eosinophilia, and an irregular pattern with thin trabeculae; occasionally, the pattern was pseudoglandular. There was replacement growth at the boundary between the tumor and the surrounding hepatic tissue. Multicentric occurrence of HCC was classified into two categories by application of the criteria used in the General Rules,<sup>19)</sup> with some modifications.<sup>7-11)</sup> In one type of occurrence (pattern 1), at least one tumor consisting of well-differentiated HCC grew in a replacement growth pattern, with hepatic structures maintained, with moderately or poorly differentiated main tumor elsewhere (near or far); a tumor embolus might be found in the portal vein of the main tumor. In the other type (pattern 2), moderately or poorly differentiated HCC contained well-differentiated HCC in all occurrences. Multiple HCCs that did not meet these criteria were assumed to be metastases originating from a main tumor; the patients without multicentric HCCs were assumed to have monocentric HCC (single HCC or HCC with metastases). The clinicopathologic criteria used were proven to be of practical use by our previous study.<sup>11)</sup>

Noncancerous hepatic tissues were also examined. The histologic activity index (HAI) score<sup>22)</sup> with some modifications<sup>23)</sup> was used to evaluate the severity of active hepatitis and the degree of fibrosis. HAI scores (components 1 to 3) of 0 indicated no activity (grading 0), 1 to 3 indicated minimal activity (grading 1), scores of 4 to 7 indicated mild activity (grading 2), scores of 8 to 11 indicated moderate activity (grading 3), and scores of 12 or greater indicated severe activity (grading 4). Component 1 indicated the degree of periportal necrosis with or without bridging necrosis and piecemeal necrosis, component 2 the degree of intralobular degeneration and focal necrosis, and component 3 the degree of portal inflammation. The

degree of fibrosis (stage) was determined by component 4 in the HAI score. Stage 1 indicated portal fibrous expansion, stage 2 portal-portal septa without architectural distortion, stage 3 portocentral septa with architectural distortion, and stage 4 cirrhosis. At least two pathologists without any knowledge of the clinical and laboratory data examined the materials.

**Statistics** The  $\chi^2$  test followed by Bonferroni's test was used to compare categorical data. Student's *t*-test was used to compare scores between groups. Differences with  $P < 0.05$  were considered significant. The odds ratio was used to estimate relative risk for multicentric HCC. For univariate analysis, logistic regression analysis was used. For multivariate analysis, multiple logistic regression analysis was used.

## RESULTS

Clinicopathologic findings for patients with and without multicentric HCCs are shown in Table I. Mean age and sex distribution did not differ between groups. Serum alanine aminotransferase (ALT) activity and serum concentration of type 4 collagen were significantly higher in the patients with multicentric HCCs than in those without them ( $P = 0.0381$ ,  $P = 0.0374$ , respectively). The serum concentration of total bilirubin tended to be higher in the patients with multicentric HCCs than in those without them ( $P = 0.0875$ ). Platelet count was significantly lower in the patients with multicentric HCCs than in those without them ( $P = 0.0117$ ). The grading score was significantly higher in the patients with multicentric HCCs than in those without them ( $P = 0.0014$ ). Although the score in component 3 did not differ between the groups, the scores in components 1 and 2 were significantly higher in the patients with multicentric HCCs than in those without them ( $P = 0.0256$ ,  $P = 0.0165$ , respectively). The staging score was significantly higher in the patients with multicentric HCCs than in those without them ( $P < 0.0001$ ).

The relationship between the prevalence of multicentric HCCs and the grading score is shown in Table II. The prevalence of multicentric HCCs increased as the grading score increased ( $P = 0.0011$ ). The prevalence was significantly higher in patients with a grading score of 3 or 4 than in those with a grading score of 0 or 1 ( $P = 0.0012$ ) and in those with a grading score of 2 ( $P = 0.0057$ ).

The relationship between the prevalence of multicentric HCCs and the staging score is shown in Table III. The prevalence of multicentric HCCs increased as the staging score increased ( $P < 0.0001$ ). The prevalence was significantly higher in patients with a staging score of 4 (cirrhosis) than in those with a staging score of 0 to 2 ( $P < 0.0001$ ). The prevalence was significantly higher in patients with a staging score of 3 than in those with a staging score of 0 to 2 ( $P = 0.0059$ ).

Table I. Profile of Hepatocellular Carcinoma (HCC) Patients with and without HCC of Multicentric Origin

	Multicentric HCC		P
	With (n=51)	Without (n=201)	
Age (years, mean±SD)	63.0±4.9	62.9±6.2	0.872
Sex (M:F) <sup>a)</sup>	46:5	167:34	0.279
Albumin (g/dl)	3.5 (3.1, 3.9)	3.6 (3.2, 4.1)	0.149
AST (IU/liter)	63 (37, 122)	61 (34, 103)	0.253
ALT (IU/liter)	79 (34, 130)	64 (31, 121)	0.0381
Total bilirubin (mg/dl)	0.9 (0.5, 1.4)	0.8 (0.5, 1.4)	0.0875
Platelets (×10 <sup>4</sup> /mm <sup>3</sup> )	10.3 (6.8, 20.3)	13.1 (7.0, 21.0)	0.0117
ICGR <sub>15</sub> (%)	18.4 (9.0, 31.2)	16.7 (8.9, 27.2)	0.230
Type 4 collagen (ng/ml) <sup>b)</sup>	8.4 (5.7, 11.0)	7.8 (5.3, 11.5)	0.0374
Grade score	2.3±0.7	2.0±0.7	0.0014
Component 1	1.7±1.4	1.2±1.3	0.0256
Component 2	2.2±1.1	1.8±1.2	0.0165
Component 3	2.8±0.8	2.6±1.0	0.137
Stage score	3.7±0.7	2.8±1.2	<0.0001

a) Number of patients.

b) Type 4 collagen could be measured in 210 patients.

Results of laboratory tests are given as medians and 10th and 90th percentiles (in parentheses). AST, aspartate aminotransferase; ALT, alanine aminotransferase; ICGR<sub>15</sub>, 15-min indocyanine green retention test.

Table II. Relationship between Hepatocellular Carcinoma of Multicentric Origin and Grading Score

Grading score	No. of patients	No. of patients with multicentric HCCs (%)
0 or 1	59	6 (10.2)
2	130	23 (17.7)
3 or 4	63	22 (36.5)

The prevalence of multicentric HCCs differs significantly among the three groups ( $P=0.0011$ ). The prevalence is significantly higher in patients with a grading score of 3 or 4 than in those with a grading score of 0 or 1 ( $P=0.0012$ ) and in those with grading score of 2 ( $P=0.0057$ ).

Table III. Relationship between Hepatocellular Carcinoma of Multicentric Origin and Staging Score

Staging score	No. of patients	No. of patients with multicentric HCCs (%)
0 to 2	79	3 (3.8)
3	52	10 (19.2)
4	121	38 (31.4)

The prevalence of multicentric HCCs differs significantly among the three groups ( $P<0.0001$ ). The prevalence is significantly higher in patients with a staging score of 4 than in those with a staging score of 0 to 2 ( $P<0.0001$ ). The prevalence is significantly higher in patients with a staging score of 3 than in those with a staging score of 0 to 2 ( $P=0.0059$ ).

Table IV. Relationship between Hepatocellular Carcinoma of Multicentric Origin and Grading Score in Patients with Staging Score of 4

Grading score	No. of patients	No. of patients with multicentric HCCs (%)
0 or 1	26	5 (19.2)
2	54	15 (27.8)
3 or 4	41	18 (43.9)

Table V. Risk Ratios for Multicentric Hepatocellular Carcinomas in Comparison to Monocentric HCCs Obtained by Univariate Analysis

Variable	Risk ratio	95% CI	P
AST (per 1 IU/liter)	1.007	0.999–1.015	0.0819
Platelet count (per 10 <sup>4</sup> /mm <sup>3</sup> )	0.927	0.871–0.986	0.008
Type 4 collagen (per 1 ng/ml)	1.129	0.978–1.305	0.0986
Grading score (per 1 score)	2.025	1.130–3.158	0.0014
Staging score (per 1 score)	2.510	1.623–3.883	<0.0001

In patients with a staging score of 4 (cirrhosis), the prevalence of multicentric HCCs tended to increase as the grading score increased ( $P=0.0780$ , Table IV). The percentage of patients with multicentric HCCs among those with a staging score of 4 and a grading score of 3 or 4 was 43.9%.

Table VI. Risk Ratios for Multicentric Hepatocellular Carcinomas in Comparison to Monocentric HCCs Obtained by Multivariate Analysis

Variable	Risk ratio	95% CI	P
Grading score (per 1 score)	1.727	1.098–2.714	0.0154
Staging score (per 1 score)	2.423	1.502–3.908	<0.0001

Risk ratios for multicentric carcinogenesis determined by univariate analysis are shown in Table V. Low platelet count, high grading score, and high staging score were significant factors. A high ALT activity and a high concentration of type 4 collagen tended to be significant risk factors. On multivariate analysis (Table VI), high grading score and high staging score were significant independent risk factors.

## DISCUSSION

In this study, we found that ALT activity was significantly higher in HCC patients with multicentric HCCs than in those without them, and that the prevalence of multicentric HCCs increased as grading score increased, indicating that the potential for carcinogenesis increased with progression of active hepatitis. Of the 3 components of grading, the scores for component 1 (periportal necrosis with or without bridging necrosis, piecemeal necrosis) and component 2 (intralobular degeneration and focal necrosis) were significantly higher in patients with multicentric HCCs than in those without them. These findings indicate that hepatocytic necrosis is closely related to multicentric carcinogenesis. Cirrhosis is known to be a premalignant condition.<sup>1)</sup> In this study, the concentration of type 4 col-

lagen, a marker for hepatic fibrosis,<sup>24, 25)</sup> and staging score were significantly higher in patients with multicentric HCCs than in those without them. The prevalence of multicentric HCCs increased as staging score increased. These findings suggest that active hepatitis and extensive fibrosis are responsible for the development of multicentric HCCs.

On univariate analysis, low platelet count, high grading score and high staging score were significant risk factors for multicentric HCCs, and high ALT activity and high concentration of type 4 collagen tended to be risk factors. High grading score and high staging score were risk factors for multicentric HCCs on multivariate analyses, indicating that these are independent risk factors. In addition, cirrhosis with active hepatitis is a strong risk factor for multicentric HCCs, and cirrhosis and active hepatitis are additive factors since about half of patients with a staging score of 4 (cirrhosis) and a grading score of 3 or 4 had multicentric HCCs.

Measurement of platelet count, serum ALT activity, and the concentration of type 4 collagen and histologic assessment of noncancerous hepatic tissue provide information useful for estimation of the potential for multicentric carcinogenesis. Careful examination to detect small nodular lesions is necessary after treatment as well as before and during treatment for HCC patients with a low platelet count, high ALT activity, high concentration of type 4 collagen, high grading score and/or high staging score.

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