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Clinicopathological Analysis of Non-B Non-C Hepatocellular Carcinoma Focusing on Cellular Proliferation

SHINJI MIZUOCHI[1], JUN AKIBA[2], REIICHIRO KONDO[1], HIRONORI KUSANO[1,3], TARO SHIOGA[1], KEIICHI KONDO[1], KANA TSUTSUI[1], MASAMICHI NAKAYAMA[1], SACHIKO OGASAWARA[1], YOSHIKI NAITO[2], OSAMU NAKASHIMA[4] and HIROHISA YANO[1]

[1]*Department of Pathology, Kurume University School of Medicine, Kurume, Japan;*
[2]*Department of Diagnostic Pathology, Kurume University Hospital, Kurume, Japan;*
[3]*Department of Clinical Laboratory, National Hospital Organization Kokura Medical Center, Kitakyushu, Japan;*
[4]*Department of Clinical Laboratory Medicine, Kurume University Hospital, Kurume, Japan*

Abstract. *Background/Aim: Non-B non-C hepatocellular carcinomas (NBNC-HCCs) are larger than hepatitis virus-related HCCs. We conducted a clinicopathological study of patients who underwent curative NBNC-HCC resection, including proliferative activity assessments, such as nuclear grade and Ki-67 labelling index (LI). Materials and Methods: Histopathological findings of 197 patients were examined, including 56 NBNC-HCCs, 45 hepatitis B virus (HBV)-related HCCs (HBV-HCC), and 96 hepatitis C virus (HCV)-related HCCs (HCV-HCC). Results: NBNC-HCCs were significantly larger than HCV-HCCs, but not significantly different from HBV-HCCs. Mitotic counts, nuclear grade, and Ki-67 LI of NBNC-HCCs were not significantly different from those of HCV-HCCs, but were significantly lower than those of HBV-HCCs. Recurrence-free survival was significantly better in the NBNC-HCC group than in the HBV-HCC group in cases with mild liver fibrosis. Conclusion: NBNC-HCCs were significantly larger in diameter, but their nuclear grade or Ki-67 LI were not significantly different from those of other HCCs, suggesting that they do not have a higher proliferative activity.*

The most important aetiological factor associated with hepatocellular carcinoma (HCC) worldwide is persistent infection with either hepatitis C virus (HCV) or hepatitis B virus (HBV). Among patients in Japan, persistent HCV infection is the leading cause of HCC (1, 2). The development of antiviral therapies has led to a relative decline in HCC caused by HCV and HBV infections. However, an increase in non-B non-C hepatocellular carcinoma (NBNC-HCC), which is negative for both HBV surface antigen (HBs Ag) and HCV antibodies (HCV Ab), is becoming a problem (3, 4).

Basic and clinical research regarding HCC has mostly focused on patients with a background of HBV- or HCV-related chronic hepatitis (5), with treatment and screening algorithms being based on these patient types. The exact background and molecular mechanisms underlying the rapid increase in the incidence of NBNC-HCC remain unclear; however, non-alcoholic steatohepatitis (NASH) and metabolic syndrome have been reported to be important risk factors (3).

Several recent studies have examined the prognosis of surgical cases of NBNC-HCC and hepatitis virus-related HCC, but the findings have been inconsistent with some showing no significant difference in prognosis, whereas others indicating that patients with NBNC-HCC have a longer disease-free survival period (6-9). A study of tumour size showed that NBNC-HCCs are larger than hepatitis virus-related HCCs (9). One possible reason for this finding is the lack of proper screening, often resulting in the diagnosis of the disease in an advanced state; moreover, research regarding the proliferative activity of HCCs is limited (10).

In the present study, we investigated why NBNC-HCCs are larger than hepatitis virus-related HCCs using patients with HCC who underwent curative resection as the initial therapy.

Materials and Methods

Patients. The study involved 197 patients with HCC who underwent hepatectomy at Kurume University Hospital, Kurume, Japan, between January 2008 and December 2013. The exclusion criteria

Correspondence to: Shinji Mizuochi, Department of Pathology, Kurume University School of Medicine, Asahi-machi 67, Kurume, 830-0011, Japan. Tel: +81 942317546, Fax: +81 942320905, e-mail: mizuochi_shinji@kurume-u.ac.jp

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were as follows: 1) previous treatment for HCC, 2) presence of multiple nodules before surgery, and 3) having autoimmune hepatitis, primary biliary cholangitis, or Japanese schistosomiasis. The clinicopathological characteristics of the 197 patients are shown in Table I. The 197 patients comprised 56 patients with NBNC-HCC (28.4%), 45 with HBV-HCC (22.8%), and 96 with HCV-HCC (48.7%). The study was approved by the Kurume University Institutional Review Board (approval No. 20116) and complied with the guidelines of the Declaration of Helsinki.

Histopathological examination. Resection specimens consisting of HCC and non-neoplastic tissue were collected from patients who underwent hepatectomy, fixed with 10% buffered formalin, and embedded in paraffin to obtain tissue blocks. The blocks were cut to sections of 4 μm thickness; then, the sections were stained with haematoxylin and eosin, and evaluated by two pathologists (SM and JA). The specimens were assessed for diameter, histological differentiation, histological subtype, portal vein invasion, intrahepatic metastatic nodules, nuclear grade, and mitotic count of the tumour as well as fibrosis, inflammation, and fatty changes in non-neoplastic tissues. Tumour subtypes were evaluated according to the World Health Organization (WHO) classification 2019 (11). Histological atypia and mitotic count were evaluated according to nuclear grade (NG), which is widely used in the pathological diagnosis of breast cancer (Figure 1, Table II) (12). NG was determined by adding nuclear atypia and mitotic count scores (12). Fibrosis and inflammation in non-neoplastic tissues were assessed according to Batts–Ludwig staging and grading (13). Fibrosis based on stage was classified as none (stage 0), mild (portal fibrosis, stage 1), moderate (periportal fibrosis, stage 2), severe (bridging fibrosis with lobar distortion, stage 3), and cirrhosis (stage 4). The patients were classified as those with mild fibrosis (F=0, F=1, or F=2) or those with severe fibrosis (F=3, or F=4). Inflammation grade according to the degree and location of the inflammation was classified as none (grade 0), minimal (grade 1), mild (grade 2), moderate (grade 3), or severe (grade 4) (13). The background liver tissue in the NBNC group was evaluated for ballooned hepatocytes and large liver cell changes (LCCs). Steatosis was defined as having ≥5% large and small lipid droplets, and steatohepatitis was defined as steatosis plus ballooned hepatocytes and lobular inflammation. Finally, NASH was defined as the absence of a history of alcohol consumption.

Tissue microarray (TMA) preparation. TMA was used to perform multiplex histological analysis of the tissue specimens. The TMA blocks were prepared using an arraying instrument (Azumaya, Tokyo, Japan). Two 4-mm diameter cores were punched from the tumour portion of the "donor" paraffin-embedded tissue block for each patient included in the study and placed onto "recipient" TMA blocks. Each TMA block contained 22 cores.

Immunohistochemical analysis. Slides were created using 4-μm thick sections cut from the TMA paraffin blocks and labelled with anti-Ki-67 antibody (NCL-Ki67-MM1, dilution 1:200; Leica Biosystems, Nussloch, Germany) using a BenchMark ULTRA slide staining system (Ventana Automated Systems Inc., Tucson, AZ, USA). The Ki-67 labelling index (LI) was calculated as the percentage of tumour cells with positively stained nuclei in the region of tissue with the highest expression of Ki-67 (hotspot).

Statistical analysis. All statistical analyses were performed using JMP Pro 13.21 software (SAS Institute Inc., Cary, NC, USA). Continuous variables are presented as mean±standard deviation. A chi-squared test or Dunnett's test was used to evaluate the discrete data. Overall survival (OS) curves and recurrence-free survival (RFS) curves were created using the Kaplan–Meier method and compared using a log-rank test. The *p*-values were corrected for multiple comparisons in all cases using Bonferroni correction following stepwise criteria. The significance level was set at $p=0.05$, and bilateral tests were performed.

Table I. *Clinicopathological characteristics of 197 patients who underwent hepatectomy for HCC.*

Clinical findings	
Number of patients	197
Age, year (average±standard deviation)	66.7±10.3 (65.2-68.1)
Gender, n (%)	
Male	152 (77.2%)
Female	45 (22.8%)
Dairy drinking, n (%)	62 (31.4%)
DM, n (%)	99 (50.5%)
HCV Ab positive, n (%)	96 (48.7%)
HBs Ag positive, n (%)	45 (22.8%)
Both HCV Ab and HBs Ag negative, n (%)	56 (28.4%)
AST (IU/l) (median, range)	35.5 (2.6-156)
ALT (IU/l) (median, range)	36.0 (7-161)
AFP (ng/ml) (median, range)	12.3 (1.1-415132)
DCP (mAU/ml) (median, range)	89 (2.65-275000)
Pathological findings	
Tumour size, mm (average±standard deviation)	35.6±26.7
Tumour differentiation	
Well	15 (7.61%)
Moderately	144 (73.1%)
Poorly	38 (19.3%)
Portal vein invasion, n (%)	119 (60.4%)
Intrahepatic metastasis, n (%)	24 (12.7%)
Fibrosis, n (stage: 0/1/2/3/4)	3/48/49/31/66
Inflammation, n (activity: 0/1/2/3)	3/87/105/2

HCC: Hepatocellular carcinoma; DM: diabetes mellitus; HCV Ab: hepatitis C virus antibody; HBs Ag: hepatitis B surface antigen; AST: aspartate transaminase; ALT: alanine aminotransferase; AFP: α-fetoprotein; DCP: des-γ-carboxy prothrombin.

Results

Comparison of clinicopathological characteristics between the NBNC group and HBV and HCV groups of patients. The clinicopathological features of the patients with NBNC-HCC, HBV-HCC, and HCV-HCC are shown in Table III. As this study focused on NBNC-HCC, we compared the NBNC-HCC and HBV-HCC groups and the NBNC-HCC and HCV-HCC groups, but not the HBV-HCC and HCV-HCC groups. Patients in the NBNC group on an average were significantly older than those in the HBV group ($p<0.001$), but comparable to those in the HCV group ($p=0.3395$). The proportion of men in the NBNC group was higher than that in the HCV group ($p=0.0104$), but similar to that in the HBV group ($p=0.5234$).

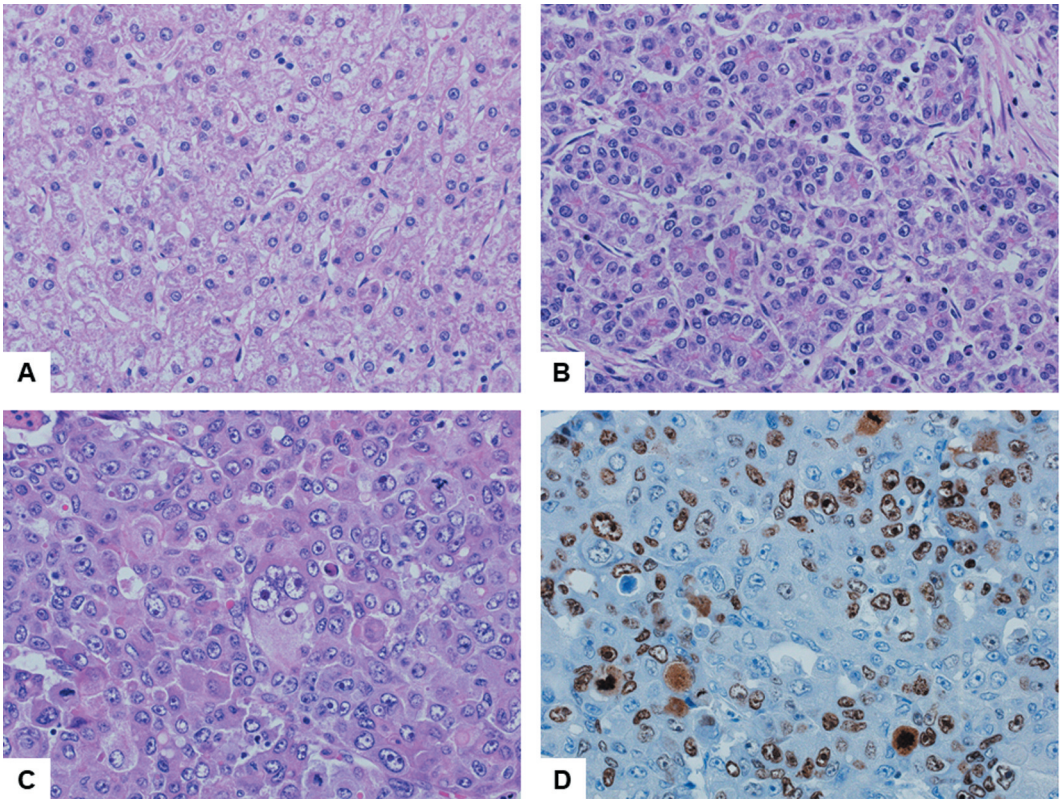

Figure 1. *Representative histologic features of nuclear atypia and immunohistochemical staining of Ki-67. (A) Nuclear atypia score 1. The tumour cells have a subtle degree of nuclear pleomorphism. (B) Nuclear atypia score 2. The tumour cells exhibit a moderate degree of nuclear pleomorphism. (C) Nuclear atypia score 3. The tumour shows a marked degree of cellular pleomorphism. Multinucleated cells are observed. (D) Immunohistochemical staining of Ki-67 revealed positive nuclear staining in approximately 50% of the tumour cells.*

The aspartate transaminase (AST) level was significantly lower in the NBNC group than in the HCV group (p=0.006). Meanwhile, the α-fetoprotein (AFP) level was significantly lower in the NBNC group than in the HBV group (p=0.0222). Portal vein invasion was significantly more common in the NBNC group than in the HCV group (p=0.0348). Finally, tumour diameter was the largest in the NBNC group at 45.2±32.7 mm, which was significantly larger than that in the HCV group (p=0.0022), but not significantly different from that in the HBV group (p=0.0575).

Among the HCC subtypes, steatohepatitis (SH)-HCC was more common in the NBNC-HCC group than in the HBV group (p=0.2552) or the HCV group (p=0.1038). Lymphocyte-rich HCC tended to be less common in the NBNC-HCC group than in the HBV group, but the difference was not significant (p=0.1720). Mitotic count scores, nuclear grade, and Ki-67 LI were similar in the NBNC and HCV groups (p=0.0888, p=1.0, and p=0.8624, respectively), but were significantly lower than those in the HBV group (p=0.0056, p=0.0334, and p=0.0031, respectively).

With respect to the background liver tissue, the NBNC group had fewer patients with severe fibrosis (≥F3) than the HBV group (p=0.0006) and milder inflammation than the HCV group (p=0.0002). The NBNC-HCC group tended to have more patients with ≥5% fatty changes than the HBV and HCV groups, but the differences were not significant (p=0.2164 and p=0.0772, respectively). The LCC was significantly less in the HBV group than in the NBNC group (p=0.0012) but significantly more in the HCV group (p=0.0014).

Characteristics of patients in the NBNC-HCC group. Of the 56 patients in the NBNC group, 26 had a history of daily alcohol consumption and 12 were positive for hepatitis-B-core-antigen antibody (HBc Ab). Steatohepatitis was diagnosed in 21 patients. Among the 21 patients, 11 patients had no history of alcohol consumption, finally defined as NASH. Eleven patients in this group had NASH with no history of alcohol consumption.

Prognosis of patients with HCC after hepatic resection. The prognosis of patients with HCC after hepatectomy is shown

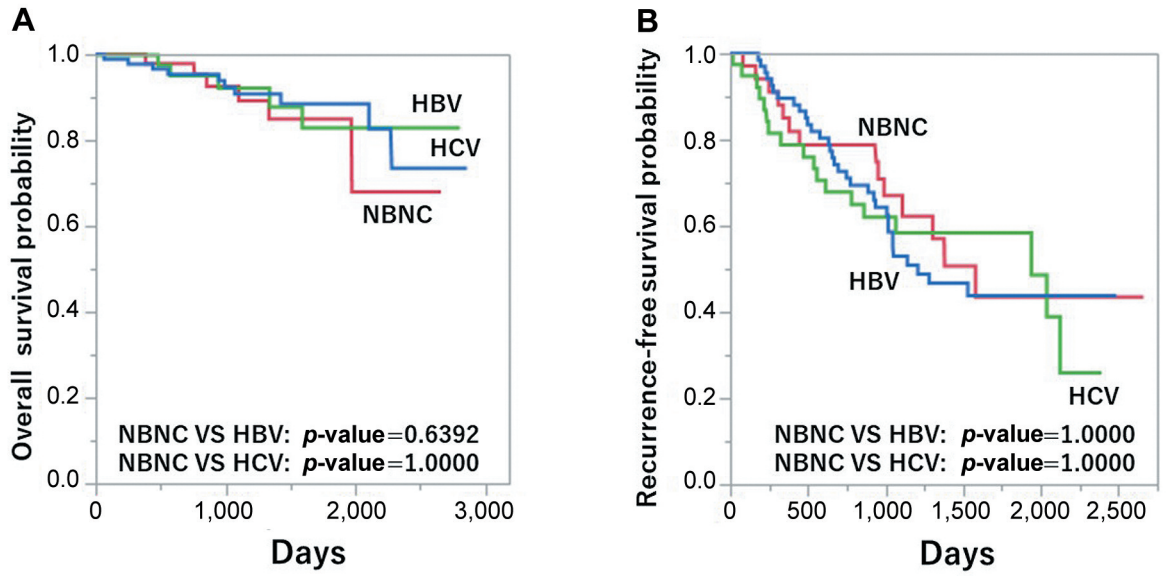

Figure 2. *Comparisons of overall survival and recurrence-free survival rates after hepatectomy among patients in the HBV–HCC, HCV–HCC, and NBNC–HCC groups. (A) No significant difference in OS was detected between the NBNC–HCC group and HBV–HCC group (p=0.6392) or the NBNC–HCC group and HCV–HCC group (p=1.0000). (B) No significant difference in recurrence-free survival was detected between the NBNC–HCC group and HBV–HCC group (p=1.0000) or the NBNC–HCC group and HCV–HCC group (p=1.0000). All comparisons were made using a log-rank test. The p-values were adjusted using the Bonferroni method (2 tests). Significance level was set at p≤0.05. HBV: Hepatitis B virus; HCV: Hepatitis C virus; NBNC: non-B non-C; HCC: hepatocellular carcinoma.*

in Figure 2 and Figure 3. No significant differences were observed between the NBNC-HCC and HBV-HCC or HCV-HCC groups regarding OS (p=0.6392 and p=1.000, respectively) or RFS (p=1.0000 and p=1.000, respectively).

Fibrosis severity in the NBNC group compared with that in the HBV and HCV groups. The severity of fibrosis in the NBNC group differed from that in the HBV and HCV groups. The RFS of patients with mild fibrosis in the NBNC-HCC group was significantly better than that in the HBV-HCC group (p=0.0368), but not significantly different from that in the HCV-HCC group (p=0.7378). No significant differences according to the degree of fibrosis were observed in OS between the NBNC-HCC group and the HBV-HCC or HCV-HCC groups.

Discussion

To investigate why patients with NBNC-HCC have larger tumours than those with HBV-HCC and HBC-HCC, we analysed the clinicopathological findings for patients with NBNC-HCC and compared them with the findings for patients with HBV-HCC and patients with HCV-HCC. The analysis included assessment of tumour proliferative activity, such as nuclear grade and Ki-67 LI.

Sex and age distribution of patients with NBNC-HCC, HBV-HCC, and HCV-HCC were similar to that previously reported for patients in Japan (4-6, 9, 14, 15). The mean HCC tumour diameter among the patients with NBNC-HCC was 45.2 mm, which was significantly larger than that among the patients with HCV-HCC, similar to the results previously reported (9, 14).

In the present study, we used two methods to investigate the potential reason for patients with NBNC-HCC to have

Table II. *Assessment of tumour nuclear grade.*

A: Nuclear grade	
Grade	Result
Grade 1	Nuclear atypia score + Mitotic count score=2 or 3
Grade 2	Nuclear atypia score + Mitotic count score=4
Grade 3	Nuclear atypia score + Mitotic count score=5 or 6
B: Nuclear atypia	
Score	Result
Score 1	Homogenous small round nuclear cells
Score 2	Score other than 1 or 3
Score 3	Multinucleated and pleomorphic cells
C: Mitotic count	
Score	Result
Score 1	<5 mitotic cells/10 HPFs
Score 2	5-10 mitotic cells/10 HPFs
Score 3	>10 mitotic cells/10 HPFs

HPFs: High power fields.

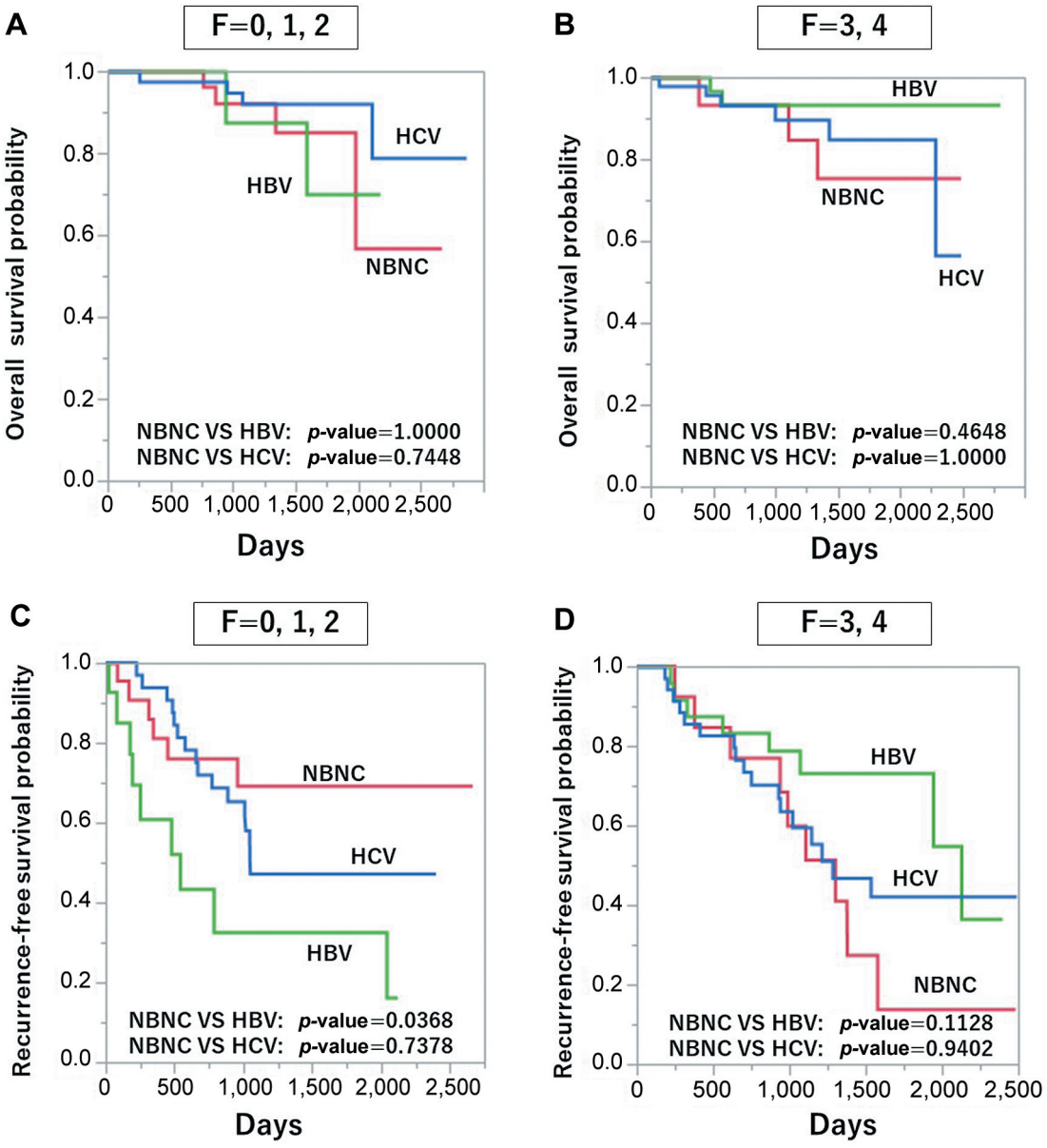

Figure 3. *Comparison of overall survival (OS) and recurrence-free survival (RFS) rates after hepatectomy between patients in the NBNC–HCC group and those in the HBV–HCC and HCV–HCC groups according to fibrosis severity in the background liver tissue. The OS and RFS were stratified by fibrosis severity into stages 0, 1, and 2 (A, C) and stages 3 and 4 (B, D). (A) OS probability of the patients with stage 0, 1, or 2 fibrosis severity. No significant difference between the NBNC–HCC group and HBV–HCC group (p=1.0000) or the NBNC–HCC group and HCV–HCC group (p=0.7448). (B) OS probability of the patients with stage 3 or 4 fibrosis severity. There was no overall significant difference between the NBNC–HCC group and HBV–HCC group (p=0.4648) or the NBNC–HCC group and HCV–HCC group (p=1.0000). (C) RFS of the patients with stage 0, 1, or 2 fibrosis severity. RFS in the NBNC-HCC group was significantly better than that in the HBV-HCC group (p=0.0368). (D) RFS with stage 3 or 4 fibrosis severity. There was no overall significant difference between the NBNC–HCC group and HBV–HCC group (p=0.1128) or the NBNC–HCC group and HCV–HCC group (p=0.9402). All comparisons were made using a log-rank test. The p-values were adjusted using the Bonferroni method (2 tests). Significance level was set as p≤0.05. HBV: Hepatitis B virus; HCV: Hepatitis C virus; NBNC: non-B non-C; HCC: hepatocellular carcinoma.*

larger tumours than those with HBV-HCC or HCV-HCC. First, we assessed nuclear atypia and mitosis based on NG, which is widely used in the pathological diagnosis of breast cancer (12). The results showed similar nuclear atypia and mitotic count scores for patients with NBNC-HCC and for those with HCV-HCC. In contrast, the HBV-HCC group had a significantly higher mean mitotic count score than the NBNC-HCC group.

Table III. *Comparison of clinicopathological characteristics among patients with NBNC-HCC, HBV-HCC, and HCV-HCC.*

	NBNC-HCC	HBV-HCC	HCV-HCC	*p*-Value*	*p*-Value**
Number of patients	56	45	96		
Clinical findings					
Age, year (average±standard deviation)	68.8±7.8	55.7±10.1	70.6±7.82	<0.001	0.3395
Gender, n (%)				0.5234	0.0104
Male	50 (89.2%)	36 (80.0%)	66 (68.8%)		
Female	6 (10.7%)	9 (20.0%)	30 (31.3%)		
DM, n (%)	30 (53.6%)	17 (37.8%)	52 (54.2%)	0.3198	1.0
AST (IU/L) (average±standard deviation)	37.6±22.3	36.9±22.3	50.0±25.8	0.9854	0.006
ALT (IU/L) (average±standard deviation)	37.8±22.4	38.8±17.9	46.0±27.7	0.9703	0.0921
AFP>100(ng/ml), n (%)	13 (23.2%)	22 (48.9%)	17 (17.7%)	0.0222	0.8166
DCP>500(mAU/ml), n (%)	22 (39.3%)	10 (22.2%)	25 (26.0%)	0.1722	0.2062
Pathological findings					
Tumour size, mm (average±standard deviation)	45.2±32.7	33.9±30.2	30.7±18.5	0.0575	0.0022
Tumour differentiation				0.8234	0.3220
Well	2 (3.6%)	1 (2.2%)	12 (12.5%)		
Moderately	45 (80.3%)	32 (71.1%)	67 (69.8%)		
Poorly	9 (16.1%)	12 (26.7%)	17 (17.7%)		
Subtype					
Conventional, n (%)	44 (78.6%)	31 (71.1%)	85 (88.5%)	0.9754	0.2134
Steatohepatic, n (%)	6 (10.7%)	1 (2.2%)	2 (2.1%)	0.2552	0.1038
Macrotrabecular massive, n (%)	5 (8.9%)	2 (4.4%)	4 (4.2%)	0.9136	0.5814
Lymphocyte-rich, n (%)	1 (1.8%)	5 (11.1%)	3 (3.2%)	0.1720	1.0
Others, n (%)	0	5 (11.1%)	2 (1.0%)	0.0308	1.0
Nuclear atypia score				0.1222	1.0
1	10 (17.9%)	3 (6.67%)	14 (14.6%)		
2	35 (62.5%)	25 (55.6%)	63 (65.6%)		
3	11 (19.6%)	17 (37.8%)	19 (19.8%)		
Mitotic count score				0.0056	0.0888
1	42 (75.0%)	19 (42.2%)	59 (61.5%)		
2	4 (7.14%)	10 (22.2%)	22 (22.9%)		
3	10 (17.9%)	16 (35.6%)	15 (15.6%)		
Nuclear grade				0.0334	1.0
1	37 (66.1%)	18 (40.0%)	56 (58.3%)		
2	8 (14.3%)	7 (15.6%)	18 (18.8%)		
3	11 (19.6%)	20 (44.4%)	22 (22.9%)		
Ki-67 Labelling index, %	8.41±15.3	15.8±19.1	7.25±11.7	0.0031	0.8624
Portal vein invasion, n (%)	39 (69.7%)	33 (73.3%)	47 (49.0%)	1.6506	0.0348
Intrahepatic metastasis, n (%)	10 (18.2%)	5 (11.3%)	9 (10.0%)	0.8158	0.4102
Fibrosis (stage: F≥3)	18 (32.1%)	31 (68.9%)	48 (50.0%)	0.0006	0.0834
Activity (activity: A≥2)	17 (30.4%)	14 (31.1%)	76 (79.2%)	1.0	0.0002
Steatosis, n (%)				0.2164	0.0772
<5%	28 (50%)	30 (66.7%)	65 (67.7%)		
≥5%	28 (50%)	15 (33.3%)	31 (33.3%)		
Large liver cell change, n (%)	20 (35.7%)	32 (71.1%)	11 (11.5%)	0.0012	0.0014

*NBNC-HCC *vs*. HBV-HCC. **NBNC-HCC *vs*. HCV-HCC. HCC: Hepatocellular carcinoma; HBV: hepatitis B virus; HCV: hepatitis C virus; DM: diabetes mellitus; AST: aspartate aminotransferase; ALT: alanine aminotransferase; AFP: α-fetoprotein; DCP: des-γ-carboxy prothrombin. All comparisons were made using a chi-squared test with Bonferroni correction or Dunnett's test. The *p*-values were adjusted using the Bonferroni method (two tests). Significance was set at $p \leq 0.05$.

Next, we examined the proliferative activity of tumour cells using Ki-67 immunostaining. Ki-67 is a marker expressed in all phases of the cell cycle except G0 (resting phase) and is widely used as a marker of proliferative activity in various tumours (11, 16, 17). Although reports regarding the Ki-67 LI in HCC vary, it has been found to correlate with high histological grade, large tumour size, and the number of tumour nodes (18). In our current study,

NBNC-HCC had a significantly larger tumour diameter than that of HCV-HCC, but its Ki-67 LI value was similar to that of HCV-HCC. This indicates that NBNC-HCC does not exhibit high proliferative activity and suggests that NBNC-HCC may be less likely to be detected early, and instead may be commonly detected in more advanced states.

The mitotic count and Ki-67 LI are high in HBV-HCC because HBV is integrated into the host DNA during the early stages of clonal expansion, which promotes genomic instability and directs insertional mutagenesis in driver genes related to carcinogenesis (19). Furthermore, the activation of p53 and cell cycle pathways are common in HBV-HCC, which may explain why the mitotic count and Ki-67 LI were higher in this group of patients than in those in the other groups (20-22).

In the current study, we also evaluated the histological subtypes of HCC. It has been reported that SH-HCC exhibits histology similar to that of steatohepatitis, including tumour-cell fat changes, ballooning, appearance of Mallory-Denk bodies, interstitial fibrosis, and infiltration of inflammatory cells (23). SH-HCC is also strongly associated with steatohepatitis and metabolic syndrome (24). Of the six patients with SH-HCC among the NBNC-HCC patients in our study, steatosis was observed in the background liver of three patients, two were with NASH. Therefore, SH-HCC was associated with steatosis/steatohepatitis, consistent with the findings of a previous study (11).

Patients with HBc Ab positivity and HBs Ag negativity are considered to have occult HBV infection (25). Of the 56 patients with NBNC-HCC in the present study, 35 were tested for HBc Ab of which 12 (34.3%) were positive. The reported frequency of HBc Ab positivity in NBNC-HCC varies from 8% to 64% (26-29). HBV DNA integration is also detected in a significantly higher proportion in HBc Ab-positive patients with HCC than in HBc Ab-negative patients with HCC (30). Furthermore, it has been reported that the HBV genome is frequently incorporated into the tumour and background liver cells of patients with NBNC-HCC, even if serum HBc Ab results are negative (28). Therefore, to histologically investigate the involvement of HBV in HCC, we evaluated the presence and absence of LCC, which is known to be closely associated with HBV infection, even though it has also been observed in various other liver disorders (28, 31-33). We detected LCC in 35.7% of the NBNC-HCC cases. Kondo *et al*. reported LCC was present in 75% of NBNC-HCC, whereas other reports indicated that LCCs are dysplastic lesions closely related to hepatocarcinogenesis or are associated with aging (34, 35). These findings demonstrate a lack of consensus. Overall, the significance of HBV in NBNC-HCC remains controversial and more detailed studies are required, including genetic analyses.

With respect to background liver tissue, many patients in our study with NBNC-HCC exhibited milder fibrosis and less inflammation than those with other types of HCC and often had ≥5% fatty changes. Fibrosis in the background liver of patients with NBNC-HCC is generally considered to be mild (9), which is consistent with our results. Among the patients in our study with NBNC-HCC, 28 (50%) exhibited ≥5% fatty changes and 11 (19.7%) had NASH or alcoholic steatohepatitis (ASH), which is similar to previously reported results from Japan (15, 36).

Several studies have investigated the prognosis of patients with NBNC-HCC after hepatectomy. The long-term prognosis of patients with NBNC-HCC is similar to that for patients with hepatitis virus-related HCC (14) and the survival rate of patients with NBNC-HCC is significantly better than that of patients with HCV-HCC, but similar to that of patients with HBV-HCC (8). Utsunomiya *et al*. reported that the RFS of patients with NBNC-HCC is significantly better than that of patients with HBV-HCC or HCV-HCC (9). We did not observe significant differences in OS or RFS between the NBNC-HCC group and the HBV-HCC or HCV-HCC group. In general, the prognosis of HCC is determined by the degree of liver preservation in the background liver tissue along with tumour-related factors (9, 37). Although tumours are relatively large in patients with NBNC-HCC, liver preservation is presumed to be maintained because of the occurrence of only mild fibrosis in the background liver. It is possible that the condition of the background liver may have negated the effects of the tumour-related factors, leading to a lack of difference in prognosis compared with other aetiologies. Among patients with mild fibrosis, RFS was significantly better in the NBNC-HCC group than in the HBV-HCC group, indicating that aggressive surgical intervention would be a good choice for NBNC-HCC patients with mild fibrosis. In contrast, RFS tended to be worse in patients with NBNC-HCC and advanced fibrosis. The precise cause of this phenomenon remains unknown. The sample size of the present study was limited and the evaluation of more cases will be needed to address this issue.

The current study had some limitations. First, this was a retrospective cohort study without prospective randomisation. Second, the medical records did not indicate the amount of alcohol consumed; thus, we did not have a precise understanding of this parameter. Third, as only patients for whom surgical resection was possible were considered, there may have been selection bias towards cases with relatively low stage disease and in which the level of liver preservation was better maintained. Finally, the effects of a preoperative diet may have improved fatty changes in patients who preoperatively had a severe fatty liver.

Conclusion

The group of patients with NBNC-HCC had significantly larger tumours than those with NBV-HCC and NCV-HCC, but they did not exhibit significant differences in nuclear

grade or Ki-67 LI, indicating the proliferative capacity was not high. The results suggest that the tumours in the NBNC-HCC group may have been detected at a more advanced state than those in the NBV-HCC and NCV-HCC groups.

Conflicts of Interest

The Authors declare that they have no conflicts of interest to disclose in relation to this manuscript.

Authors' Contributions

Shinji Mizuochi, Reiichiro Kondo and Jun Akiba conceived the study. Shinji Mizuochi and Jun Akiba conducted pathological assessment. Shinji Mizuochi, Reiichiro Kondo, Hironori Kusano, Taro Shioga, Keiichi Kondo, Kana Tsutsui, Masamichi Nakayama, Sachiko Ogasawara, Yoshiki Naito and Osamu Nakashima participated in pathological diagnosis. Shinji Mizuochi and Reiichiro Kondo collected and analyzed the data. Shinji Mizuochi and Jun Akiba drafted the article. All Authors have read and approved the final version of the manuscript.

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