



Pathological prognostic factors for post-resection survival in patients with hepatocellular carcinoma associated with non-alcoholic fatty liver disease

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Background: The incidence of hepatocellular carcinoma (HCC) in patients with non-alcoholic fatty liver disease (NAFLD) is steadily increasing. However, little is known about the characteristics of these patients or the factors affecting their prognosis. Our aim was to evaluate the pathological prognostic factors associated with survival in NAFLD patients.

Methods: This was a retrospective cohort study of 575 patients who underwent resection of HCC between January 2004 and December 2018. HCC was associated purely with NAFLD or hepatitis B virus (HBV) based on the pathology and viral markers. The pathological markers of HCC were compared between patients with pure NAFLD and patients with pure HBV.

Results: The pathological factors were similar between the two groups. There were no differences in overall survival (OS; $P=0.283$) or recurrence-free survival (RFS; $P=0.990$) between the pure NAFLD and pure HBV groups. The NAFLD group had a similar local RFS ($P=0.785$) but a better systemic RFS compared with the HBV group, ($P=0.089$). In multivariable analysis using bootstrapping with resampling and replacement of data, no single factor was significantly associated with RFS. However, the Ki-67 labeling index [$P=0.022$; bootstrap 95% confidence interval (CI): 0.000–0.919] was the only independent factor associated with systemic recurrence in the NAFLD group.

Conclusions: In patients with HCC associated with NAFLD, the Ki-67 labeling index determined by immunohistochemistry may predict the risk of systemic recurrence following resection, allowing for stricter monitoring protocols for such patients.

Keywords: Ki-67; non-alcoholic fatty liver disease (NAFLD); hepatocellular carcinoma (HCC); p53; survival

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Introduction

The advances provided by science and technology have also introduced some pitfalls and problems. In particular, there have been profound changes in the dietary habits of people worldwide, which have led to an epidemic of obesity and its co-morbidities, including metabolic syndrome and type 2 diabetes (1,2). Non-alcoholic fatty liver disease (NAFLD) is becoming rampant, and its incidence is increasing in parallel with the increasing incidences of type 2 diabetes and metabolic syndrome (3,4). No effective pharmacological therapy has been found for the treatment of steatohepatitis (5). NAFLD has the potential to progress to hepatocellular carcinoma (HCC). Although HCC is commonly associated with hepatitis B virus (HBV) and hepatitis C virus (HCV), in recent years there has been a steady increase in the number of patients diagnosed with HCC associated with NAFLD. Currently, little is known about the characteristics of these patients or the factors that affect their prognosis. Therefore, it has become imperative to identify the factors associated with poor prognosis of HCC in patients with NAFLD. The criteria used to diagnose NAFLD are mainly based on pathological and clinical features (6). HCC is characterized by distinct pathological findings and immunohistochemical features. Some of the factors previously identified as prognosticators for HCC are serum AFP level, des-gamma-carboxy prothrombin level, tumor size and number, margin status, major vessel invasion, tumor stage, Edmonson-Steiner (ES) grade, Child-Pugh score, portal hypertension and cirrhosis. Immunohistochemical markers studied are keratin 19 (K19), epithelial cell adhesion molecule (EpCAM), and CD133 (7). No previous studies have shown the influence of the etiology of HCC on the risk for recurrence after resection. Therefore, we sought to determine the prognostic pathological factors for post-resection survival in patients with HCC associated with NAFLD.

We present the following article in accordance with the STROBE reporting checklist (available at <https://dx.doi.org/10.21037/tcr-21-707>).

Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This retrospective analysis of patients who underwent surgical resection for HCC was conducted after approval from the institutional review board at Seoul National University Bundang Hospital (South Korea) (IRB No. B-2007/625/107). Individual consent for

this study was waived. After pathological review, patients diagnosed with pure NAFLD or pure HBV were eligible for this study. NAFLD was diagnosed based on the scores of steatosis, lobular inflammation, and hepatocyte ballooning. Steatosis was scored as 0, 1, 2, and 3 based on the observed macrovesicular steatosis (<5% =0, ≥5% to <33% =1, ≥33% to <66% =2, and ≥66% =3). Lobular inflammation was scored as 0, 1, 2, or 3 if 0, <2, 2–4, or ≥4 foci, respectively, were detected per field (magnification, 200×). Hepatocyte ballooning was graded as 0, 1, or 2 if there were none, a few, or many ballooned hepatocytes, respectively. The NAFLD activity score was calculated as the sum of these three scores (total score, 0–8 points) for each patient (6). A score of ≥3 was classified as non-alcoholic steatohepatitis (8). Pure NAFLD was defined as alcohol intake <20 g/day and no evidence of viral hepatitis (9). Pure HBV was defined as pathologic confirmation of chronic hepatitis together with viral markers for HBV, in the absence of alcoholic intake (i.e., <20 g/day), other viral causes of hepatitis, and NAFLD.

The demographic characteristic (age, body mass index, and sex), preoperative factors [American Society of Anesthesiologists (ASA) score, co-morbidities, preoperative tumor size, and number of tumors], intraoperative findings (operation time and blood loss), postoperative factors (complications and hospital stay), and pathological factors [cirrhosis, margin status, microvascular invasion, serosal invasion, bile duct invasion, T staging, ES grade, and immunohistochemistry findings for cyclooxygenase-2 (COX2), Ki-67, and p53] were retrieved from medical records.

Pathological evaluation

All specimen slides were evaluated by two pathologists. The background liver was evaluated to confirm the presence of NAFLD or HBV. The presence and type of cirrhosis were evaluated microscopically. The tumor was then evaluated using gross and microscopic sections. Characteristics, including microvascular invasion, serosal invasion, and bile duct invasion were evaluated. The ES grade was noted and the margin status was evaluated. Immunohistochemistry was performed using 4-μm-thick paraffin-embedded sections. Sections were stained with specific antibodies for p53 (DO-7, 1:1,000, Dako), Ki-67 (MIB-1, 1:200, Dako), and COX2 (COX229, 1:300, Invitrogen) using an automated immunostainer (BenchMark XT, Ventana Medical Systems, Tucson, AZ, USA) in accordance with the manufacturer's

protocol. For Ki-67, cells with any intensity of nuclear staining were considered positive whereas only strong nuclear staining was considered positive for p53 (10). For COX2, cytoplasmic staining was considered positive. After counting 500–1,000 cells per slide, the percentages of hepatocytes positive for each antibody were calculated.

Statistical analysis

Data were tabulated on an Excel (Microsoft, Redmond, WA, USA) spreadsheet and statistical analyses were performed using SPSS for Windows version 20 (IBM, Chicago, IL, USA). Categorical data are presented as frequencies and percentages, and continuous data are presented as the mean \pm standard deviation. Between-group comparisons were conducted using logistic regression analysis. Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test. All tests were two-tailed and a P value of <0.05 was considered significant. For the survival analysis of patients in the NAFLD group, a P value of <0.1 was considered significant, this was due to the fact that very few factors showed a significance level of <0.05 so could not be used for multivariate analysis. Overall survival (OS) was calculated as the time from the date of surgery to the date of the last follow-up or death. Recurrence-free survival (RFS) was calculated as the time from the date of surgery to the date of recurrence or date of last follow-up whichever is earlier. Local RFS was calculated as the time from the date of surgery to the date of hepatic recurrence. Systemic RFS was calculated as the time from the date of surgery to the date of the first instance of extrahepatic progression. Prognostic factors in patients with NAFLD were validated using Cox regression analysis and a bootstrap procedure (2,000 times). Bootstrap validation is a method of random resampling with replacement from an original dataset for assessing statistical inference (11).

Results

Of 857 patients who underwent liver resection for HCC in the study period, 575 were eligible for this study. The pure NAFLD group comprised 36 (6.3%) patients and the pure HBV group comprised 539 (93.7%) patients.

Pathological characteristics

The pathological characteristics of both groups are displayed in *Table 1*. Cirrhosis was present in both groups

of patients, and the majority of patients in the NAFLD group had a mixed type of cirrhosis ($n=17$, 89.5%) and none had pure micronodular cirrhosis ($P=0.039$). Gross pathology revealed an expanding nodular type in half of the patients in each group, and none of the patients in the NAFLD group showed evidence of the gross infiltrative or trabecular types. Both groups were similar in terms of the proportions of patients with benign findings ($P=0.998$) and a positive resection margin ($P=0.462$). The majority of patients in both groups had a solitary tumor ($P=0.679$) with similar numbers of patients with small tumors (<2 cm). Furthermore, there were no differences in the proportions of patients with satellite nodules ($P=0.786$), microvascular invasion ($P=0.359$), and serosal invasion ($P=0.635$). The ES tumor grade ($P=0.806$) and the pathological T stage ($P=0.745$) were also similar in both groups. In terms of immunohistochemistry findings, there were no differences in p53 ($P=0.892$), COX2 ($P=0.784$), and Ki-67 ($P=0.548$) expression levels between the two groups (*Table 1*).

Factors affecting survival

The patients were followed up for a median of 42 [21–75] months. There was no difference in the OS between the pure NAFLD and the pure HBV group ($P=0.283$). However, the systemic RFS was greater in the pure NAFLD group compared to the pure HBV group, ($P=0.089$, *Figure 1*). There was no difference in the overall RFS ($P=0.925$) and local RFS ($P=0.877$) between both groups (*Figure 2*). The 5-year OS and RFS was 84.6% and 42% in the NAFLD group versus 75.3% and 41.8% in the HBV group respectively. In the univariate analyses of all patients with HCC, pathological factors associated with 5-year RFS were cirrhosis ($P=0.008$), positive resection margin ($P=0.004$), microvascular invasion ($P<0.001$), serosal invasion ($P=0.002$), ES grade ($P=0.030$), T stage ($P<0.001$), and Ki-67 $>15\%$ ($P=0.001$) (*Table 2*). In the multivariable analysis, positive resection margin [OR: 2.047, 95% confidence interval (CI): 1.226–3.416], T stage ($P=0.002$; OR: 1.815, 95% CI: 1.255–2.626), microvascular invasion ($P<0.001$; OR: 1.794, 95% CI: 1.375–2.340), and Ki-67 $>15\%$ ($P=0.048$; OR: 1.310, 95% CI: 1.002–1.713) were associated with 5-year RFS (*Table 3*).

The pathological factors associated with 5-year OS were microvascular invasion ($P<0.001$), serosal invasion ($P=0.003$), ES grade ($P=0.016$), T stage ($P<0.001$), and Ki-67 $>15\%$ ($P<0.001$) (*Table 2*). The factors associated with 5-year OS in the multivariable analysis were T stage ($P=0.015$; OR: 1.867, 95% CI: 1.128–3.091), microvascular

Table 1 Pathological characteristics of patients in the HBV and NAFLD groups

| Characteristics | HBV, n (%) | NAFLD, n (%) | P value |
|------------------------|----------------|--------------|---------|
| Cirrhosis | 335 (62.2) | 19 (52.8) | 0.265 |
| Type of cirrhosis | | | 0.039 |
| Micronodular | 18 (5.4) | 0 | |
| Macronodular | 82 (24.5) | 1 (5.3) | |
| Mixed | 218 (40.4) | 17 (89.5) | |
| Incomplete | 16 (4.8) | 1 (5.3) | |
| Satellite nodules | 51/525 (9.7) | 3 (8.3) | 0.786 |
| Gross type | | | 0.145 |
| Expanding nodular | 257/488 (52.7) | 24/36 (66.7) | |
| Multinodular confluent | 203/488 (41.6) | 12/36 (33.3) | |
| Infiltrative | 15/488 (3.1) | 0 | |
| Trabecular | 5/588 (1.0) | 0 | |
| Resection margin | | | 0.462 |
| R0 | 508 (94.2) | 35 (97.2) | |
| R1 | 31 (5.8) | 1 (2.8) | |
| Major vessel invasion | 38 (7.1) | 4 (11.1) | 0.369 |
| Bile duct invasion | 38 (7.1) | 4 (11.1) | 0.369 |
| Microvascular invasion | 237 (44.0) | 13 (36.1) | 0.359 |
| Serosal invasion | 139 (25.8) | 8 (22.2) | 0.635 |
| Solitary tumor | 466 (86.5) | 32 (88.9) | 0.679 |
| Tumor size ≤ 2 cm | 136 (25.2) | 10 (27.8) | 0.734 |
| ES grade | | | 0.806 |
| 1+2 | 190/508 (37.4) | 12/34 (35.3) | |
| 3+4 | 318/508 (62.6) | 22/34 (64.7) | |
| T stage | | | 0.745 |
| 1+2 | 469 (87.0) | 32 (88.9) | |
| 3+4 | 70 (13.0) | 4 (11.1) | |
| Recurrence | 389 (53.6) | 19 (52.8) | 0.922 |
| Local recurrence | 266 (49.4) | 16 (44.4) | 0.569 |
| Systemic recurrence | 123 (22.8) | 3 (8.3) | 0.054 |
| p53 | | | 0.892 |
| <25% | 42/428 (9.8) | 3/28 (10.7) | |
| >25% | 107/428 (25.0) | 6/28 (21.4) | |

Table 1 (continued)

Table 1 (continued)

| Characteristics | HBV, n (%) | NAFLD, n (%) | P value |
|-----------------|----------------|--------------|---------|
| COX2 | | | 0.784 |
| <25% | 25/248 (10.1) | 0 | |
| >25% | 184/248 (74.2) | 6 (16.7) | |
| Ki-67 | | | 0.548 |
| <15% | 273/438 (62.3) | 21/31 (67.7) | |
| >15% | 165/438 (37.7) | 10/31 (32.3) | |

Values are n (%). HBV, hepatitis B virus; NAFLD, non-alcoholic fatty liver disease; ES, Edmonson-Steiner; COX2, cyclooxygenase-2.

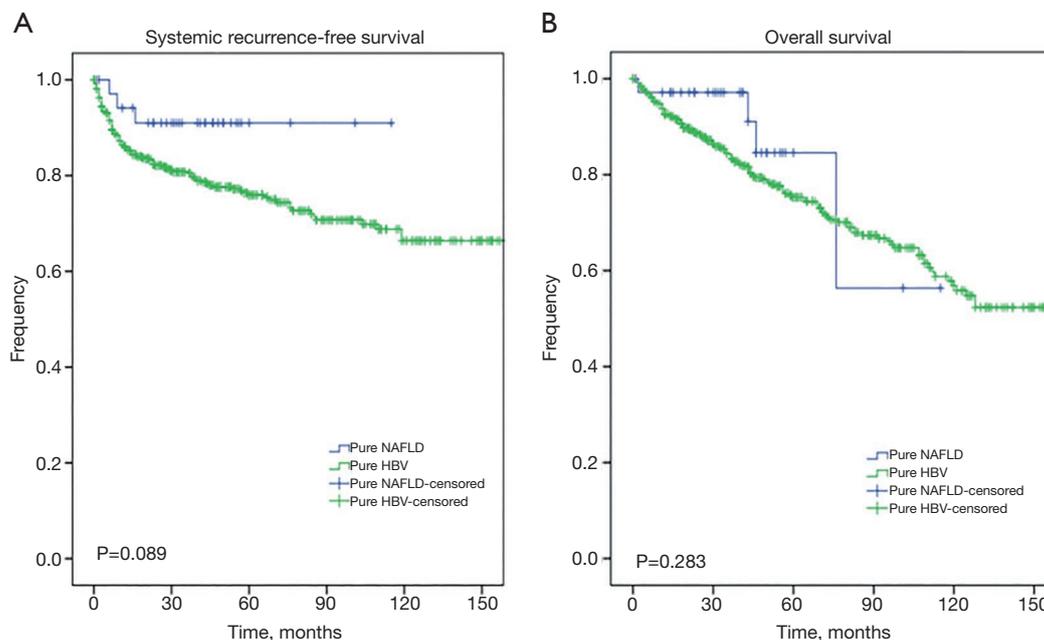


Figure 1 Systemic RFS (A) and OS (B) curves for patients with HCC associated purely with NAFLD or HBV. RFS, recurrence-free survival; OS, overall survival; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; HBV, hepatitis B virus.

invasion (P=0.003; OR: 1.861, 95% CI: 1.227–2.823), and Ki-67 >15% (P=0.023; OR: 1.586, 95% CI: 1.065–2.361) (Table 3).

Factors affecting survival in patients with NAFLD

On analyzing NAFLD patients for pathological factors affecting survival, cirrhosis (P=0.084), ES grade (P=0.054), T stage (P=0.019), Ki-67 >15% (P=0.071), and p53 (P=0.038) were associated with 5-year RFS, but no pathological factors were associated with 5-year OS. After performing multivariable analysis using bootstrapping with

resampling and replacement of data up to 2,000 times, none of the variables were significantly associated with RFS.

Recurrence was then divided into local recurrence and systemic recurrence (Table 4). None of the variables were significantly associated with local RFS. However, for systemic RFS, the univariate analyses revealed that hypertension (P=0.067), serosal invasion (P=0.085), T stage (P<0.001) and Ki-67 (P=0.021) were significant variables (Figure 3). In the multivariable analysis with the bootstrapping method, Ki-67 (P=0.022; bootstrap 95% CI: 0.000–0.919) was the only independent variable associated with systemic recurrence in patients with NAFLD.

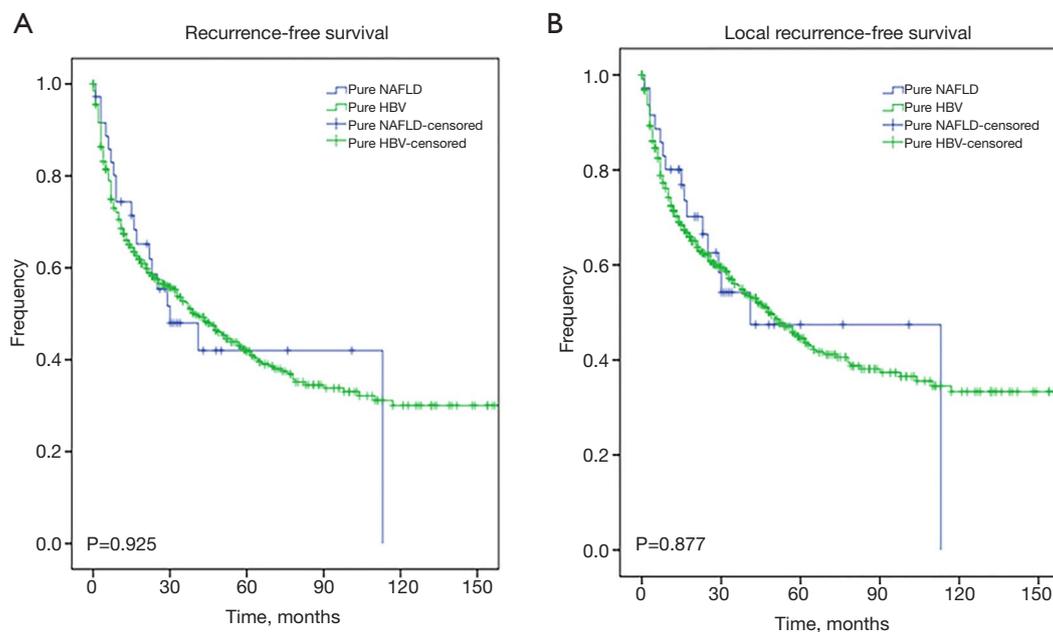


Figure 2 Overall RFS (A) and local RFS (B) curves for patients with HCC associated purely with NAFLD or HBV. RFS, recurrence-free survival; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; HBV, hepatitis B virus.

Discussion

HCC is the second leading cause of cancer-related deaths worldwide (12). HCC commonly occurs in patients with viral hepatitis (13). However, with recent increase in the incidence of NAFLD, the number of patients with HCC associated- NAFLD has also increased. Various methods, including systemic agents, radiofrequency ablation, transarterial chemoembolization, microwave ablation, radiotherapy, surgical resection, and liver transplantation are available for the treatment of HCC (14,15). Surgical resection is the preferred treatment considered curative for HCC (16). HCC has a distinct set of histological and immunological characteristics (7). Various studies have analyzed the impact of molecular markers on the prognosis of HCC (17-19), but there is no data regarding the prognostic relevance of these molecular markers in patients with HCC associated with NAFLD.

The frequency of cirrhosis was similar in the NAFLD and HBV groups in this study, and the development of cirrhosis is not critical for the development of HCC in patients with NAFLD (20). None of the patients in the NAFLD group had a purely micronodular pattern of cirrhosis; instead, the mixed pattern was the most common pattern found. Other pathological variables, including the ES grade, microvascular invasion, and serosal invasion,

were similar in both groups. Furthermore, there were no differences in the immunohistochemistry markers between the groups. Of note, the 5-year RFS and OS rates were similar in both groups. While the 5-year local RFS rate was similar in both groups, the systemic RFS was better in the NAFLD group. Microvascular invasion and Ki-67 >15% were identified as prognostic factors associated with RFS in patients with HCC. In addition to these factors T stage was associated with OS. However, there were no independent prognostic factors in patients with NAFLD. When we analyzed local and systemic RFS, we found that Ki-67 was the only independent prognostic pathological factor associated with systemic RFS.

Ki-67 is a protein named after the city in which it was first discovered (Kiel) and the number of the original clone. It was initially believed to be an antigen but was later identified as a protein (21). Positivity for Ki-67 is a measure of cellular growth because it is expressed during active growth phases and absent in the rest phase (22). The Ki-67 labeling index, defined as the percentage of cells positive for Ki-67, provides a useful insight into the growth characteristics of the tumor as well as the potential outcome of patients because it represents the physiological status of cellular proliferation (23). A recent meta-analysis revealed that Ki-67 is strongly associated with the aggressiveness of

Table 2 Univariate analyses of factors associated with survival in patients with HCC

| Characteristics | N (%) | RFS | OS |
|-------------------------------|----------------|--------|--------|
| Age >65 years | 126 (21.9) | 0.719 | 0.005 |
| Male sex | 447 (77.7) | 0.025 | 0.717 |
| Heart disease | 20 (3.5) | 0.553 | 0.211 |
| Hypertension | 171 (29.7) | 0.339 | 0.226 |
| DM | 108 (18.8) | 0.842 | 0.028 |
| Hyperlipidemia | 35 (6.1) | 0.378 | 0.211 |
| Obesity | 243 (42.3) | 0.011 | 0.002 |
| Child-Pugh B or C | 29 (5.0) | 0.119 | 0.062 |
| MELD >10 | 63 (11.0) | 0.581 | 0.096 |
| AFP >20 ng/mL | 253 (44.0) | 0.009 | 0.006 |
| Hypoalbuminemia | 62 (10.8) | 0.005 | 0.051 |
| Thrombocytopenia | 82 (14.3) | 0.017 | 0.001 |
| Major liver resection | 142 (24.7) | 0.143 | <0.001 |
| Anatomical resection | 342 (59.5) | 0.761 | 0.175 |
| Operation time \geq 200 min | 382 (66.4) | 0.094 | 0.135 |
| Blood loss >500 mL | 213 (37.0) | <0.001 | 0.003 |
| Blood transfusion | 106 (18.4) | 0.008 | 0.002 |
| Cirrhosis | 354 (61.6) | 0.008 | 0.101 |
| R0 | 32 (5.6) | 0.004 | 0.103 |
| Microvascular invasion | 250 (43.5) | <0.001 | <0.001 |
| Serosal invasion | 147 (25.6) | 0.002 | 0.003 |
| ES grade | | 0.030 | 0.016 |
| 1+2 | 202/542 (37.3) | | |
| 3+4 | 340/542 (62.7) | | |
| T stage | | <0.001 | <0.001 |
| 1+2 | 501 (87.1) | | |
| 3+4 | 74 (12.9) | | |
| Major complications | 68 (11.8) | 0.174 | 0.152 |
| HBV | 539 (93.7) | 0.925 | 0.283 |
| NAFLD | 36 (6.3) | – | – |
| Ki-67 >15% | 175 (30.4) | 0.001 | <0.001 |
| COX2 >25% | 190/284 (66.9) | 0.722 | 0.393 |
| p53 >25% | 113/456 (24.8) | 0.166 | 0.336 |

HCC, hepatocellular carcinoma; RFS, recurrence-free survival; OS, overall survival; DM, diabetes mellitus; MELD, Model for End-Stage Liver Disease; AFP, α -fetoprotein; ES, Edmonson-Steiner; COX2, cyclooxygenase-2.

Table 3 Multivariable analyses of factors associated with survival in patients with HCC

| Characteristics | P value RFS | OR (95% CI) | P value OS | OR (95% CI) |
|------------------------|-------------|---------------------|------------|---------------------|
| Age >65 years | – | – | 0.001 | 2.073 (1.343–3.200) |
| Sex | 0.020 | 0.670 (0.478–0.940) | – | – |
| DM | – | – | 0.368 | 0.759 (0.417–1.383) |
| Obesity | 0.087 | 0.790 (0.604–1.034) | 0.065 | 0.671 (0.439–1.025) |
| Thrombocytopenia | 0.362 | 1.186 (0.822–1.711) | <0.001 | 2.704 (1.690–4.326) |
| AFP >20 ng/mL | 0.204 | 1.187 (0.911–1.548) | 0.105 | 1.403 (0.931–2.114) |
| Major resection | – | – | 0.401 | 1.209 (0.777–1.880) |
| Blood loss >500 mL | 0.260 | 1.195 (0.877–1.630) | 0.939 | 0.981 (0.605–1.590) |
| Transfusion | 0.482 | 1.141 (0.790–1.647) | 0.128 | 1.462 (0.897–2.383) |
| R0 | 0.006 | 2.047 (1.226–3.416) | – | – |
| T stage | 0.002 | 1.815 (1.255–2.626) | 0.015 | 1.867 (1.128–3.091) |
| Cirrhosis | 0.110 | 1.259 (0.949–1.668) | – | – |
| Microvascular invasion | <0.001 | 1.794 (1.375–2.340) | 0.003 | 1.861 (1.227–2.823) |
| Serosal invasion | 0.915 | 0.984 (0.732–1.323) | 0.865 | 1.037 (0.681–1.580) |
| ES grade | 0.944 | 1.010 (0.761–1.341) | 0.483 | 1.171 (0.753–1.822) |
| Ki-67 >15% | 0.048 | 1.310 (1.002–1.713) | 0.023 | 1.586 (1.065–2.361) |

HCC, hepatocellular carcinoma; RFS, recurrence-free survival; OR, odds ratio; CI, confidence interval; OS, overall survival; DM, diabetes mellitus; AFP, α -fetoprotein; ES, Edmonson-Steiner.

the tumor. A high Ki-67 labeling index was associated with the potential deterioration of patients with HCC (24). In contrast to our findings, El-Bendary *et al.* reported that the Ki-67 proliferation index was greater in patients with HBV than in patients with NAFLD (22). However, Hussein *et al.* reported that the proliferation index was similar in patients with NAFLD and patients with HCV (25).

Several studies have shown that the Ki-67 labeling index is a prognostic factor for recurrence and OS in patients with HCC (26,27). However, most of these earlier studies included a mixed population. The molecular markers for prognosis may differ between the different etiologies of HCC, including HBV, HCV, NAFLD, and alcoholic liver disease. Hence, studies involving mixed populations may provide inaccurate results (28). Hence we decided to study Ki-67 as a predictor for patients with NAFLD associated HCC and did not analyze patients in the HBV group in this respect. Although Ki-67 appeared to have prognostic roles for OS and RFS in our analyses of all patients with HCC, this was not apparent in the analyses of patients with pure NAFLD. The systemic recurrence rate was much lower

in the NAFLD group than in the HBV group. When we studied NAFLD patients in detail, what was interesting to note was that even though Ki-67 was not significantly related to OS and RFS, it was found to be a prognostic marker for systemic recurrence.

The serum p53 levels is another marker associated with poor prognosis in patients with HCC (29) and studies have demonstrated the association of p53 with RFS (30). HCCs with mutated p53 show high malignant potential, and p53 mutations in the primary lesion can indicate the biological behavior of recurrent HCCs. It is also a useful independent prognostic factor associated with survival after recurrence (31). In a recent prospective study, p53 overexpression was the most significant factor associated with OS after resection of HCC, and was more significant than other factors such as tumor size, vascular invasion, and tumor capsule invasion, which were also associated with OS (32). In the present study, however, we could not detect a prognostic role of p53 for survival in patients with NAFLD.

The retrospective design of this study and the limited sample size, especially of patients with pure NAFLD, are

Table 4 Factors associated with survival in patients with HCC associated with NAFLD

| Characteristics | Local RFS | Systemic RFS |
|-------------------------|-----------|--------------|
| Age >65 years | 0.501 | 0.544 |
| Male sex | 0.220 | 0.119 |
| Heart disease | 0.453 | 0.513 |
| Hypertension | 0.515 | 0.067 |
| DM | 0.410 | 0.789 |
| Hyperlipidemia | 0.203 | 0.760 |
| Obesity | 0.110 | 0.920 |
| Child-Pugh | 0.735 | 0.715 |
| MELD >10 | 0.956 | 0.600 |
| AFP >20 ng/mL | 0.986 | 0.473 |
| Hypoalbuminemia | 0.796 | 0.338 |
| Thrombocytopenia | 0.920 | 0.659 |
| Major resection | 0.852 | 0.263 |
| Anatomical resection | 0.178 | 0.459 |
| Operation time ≥200 min | 0.402 | 0.322 |
| Blood loss >500 mL | 0.414 | 0.877 |
| Blood transfusion | 0.627 | 0.355 |
| Cirrhosis | 0.117 | 0.375 |
| R0 | 0.428 | 0.715 |
| Microvascular invasion | 0.997 | 0.415 |
| Serosal invasion | 0.696 | 0.085 |
| ES grade | 0.185 | 0.106 |
| T stage | 0.158 | <0.001 |

HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; RFS, recurrence-free survival; DM, diabetes mellitus; MELD, Model for End-Stage Liver Disease; AFP, α -fetoprotein; ES, Edmonson-Steiner.

the major limitations of this study. However, even though number of patients with NAFLD associated HCC are increasing, the overall number is not very high world over and a timely evaluation of outcomes of NAFLD associated HCC was required hence we went ahead with our evaluation. To the best of our knowledge, however, this study is the first of its kind to evaluate the pathological prognostic factors in patients with NAFLD. Cirrhosis is an important limiting factor in the study leading to bias. Since evaluation of patients with NAFLD and cirrhosis reduced the number of patients

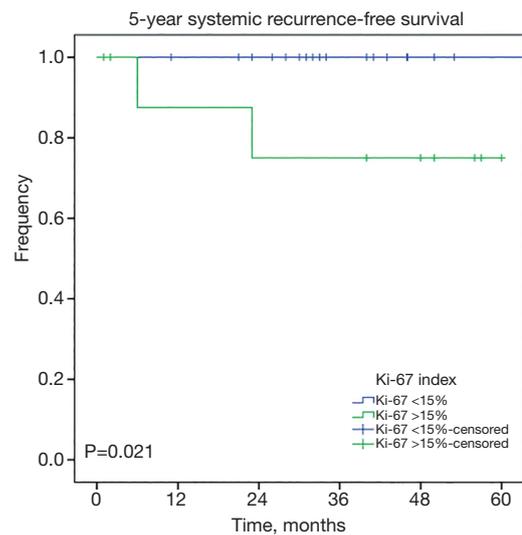


Figure 3 Systemic RFS curves for patients with NAFLD divided by the Ki-67 labeling index (<15% or >15%). RFS, recurrence-free survival; NAFLD, non-alcoholic fatty liver disease.

further, we were not able to accurately analyze this subgroup. Further studies on this topic are necessary to validate our results. In particular, a large-scale multicenter study will provide greater value in this setting.

Conclusions

HCC in patients with NAFLD can occur even in the absence of cirrhosis. Molecular markers are important to predict the survival of patients with HCC. In patients with HCC associated with NAFLD, the Ki-67 labeling index determined by immunohistochemistry may help to predict the risk of post-resection extrahepatic recurrence, and allow for stricter monitoring protocols for such patients.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional review board at Seoul National University Bundang Hospital (South Korea) (IRB No. B-2007/625/107) and individual consent for this retrospective analysis was waived.

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