of low-grade inflammation and a low serum AST level may indicate the end stage of cirrhotic NASH. Accordingly, the risk factors for the development of HCC in patients with NASH are the features of end-stage NASH and older age.

We compared the clinical features of 34 NASH-related HCC (NASH-HCC) patients and 56 age-, sex-, and treatment-matched patients with hepatitis C virus infection-related HCC (HCV-HCC).[32] As expected, there was a significantly higher prevalence of obesity, diabetes, and dyslipidemia in the NASH-HCC group. Serum transaminases were significantly higher in the HCV-HCC group, while the serum gamma-glutamyl transferase level was significantly higher in the patients with NASH-HCC. The 5-year survival rate was 55.2%, and the 5-year recurrence rate after curative treatment was 69.8% in patients with NASH-HCC. The survival and recurrence rates were similar in the two groups. HCC in NASH may also be of multicentric origin, similar to the case of HCC associated with viral hepatitis.

According to previous studies, 10–75% of all NASH-related HCCs occur in patients with non-cirrhotic NASH.[33] The high incidence of HCC arising from non-cirrhotic NASH may be partly due to the fact that the diagnosis of NASH is based on histology, and liver tissue can only be obtained by liver biopsy or surgery in patients with preserved liver function. Moreover, end-stage cirrhotic NASH cannot be diagnosed with any confidence because of its "burned out" histology. These points may introduce significant bias. Further studies are required to clarify the true incidence of HCC arising from non-cirrhotic NASH.

How is NAFLD/NASH diagnosed?

The diagnosis of NAFLD is based on the presence of the following three criteria: non-alcoholic, detection of steatosis either by imaging or by histology, and appropriate exclusion of other liver diseases.[1–6] NASH is diagnosed based on the presence of steatohepatitis on liver biopsy. Given the lack of surrogate markers yet for the diagnosis of NAFLD, it is important to exclude other liver diseases such as alcoholic liver diseases, viral hepatitis, autoimmune liver diseases, and metabolic or hereditary liver diseases. However, the prevalence of NAFLD is extremely high, NAFLD is often complicated by other liver diseases such as viral hepatitis, etc., and NAFLD exacerbates liver damage and reduces the response to treatments. Epidemiological studies have shown that alcoholic liver disease can occur when the daily alcohol consumption exceeds 20 g in women and 30 g in men. Then, NAFLD is diagnosed when the alcohol consumption is lower than the aforementioned in the respective sexes. Serum transaminases are not helpful for the diagnosis of steatosis because 50–80% of patients with hepatic steatosis have normal transaminase levels. In stage 3 fibrosis, fibrosis markers such as hyaluronic acid, etc., are elevated, and in the cirrhotic stage, reduction of the platelet count and evidence of liver dysfunction such as elevation of the serum bilirubin and ammonia, etc., are noted.

Imaging modalities. Abdominal US is currently the most common method employed for qualitative assessment of hepatic steatosis because it is non-invasive, widely available, cheap, and provides useful information. Presence of hepatic steatosis on abdominal US is usually defined based on the presence of at least two of the following findings: increased hepatorenal contrast, liver brightness, deep attenuation, and vascular blurring (Fig. 5). However, the diagnosis by US has several limitations; it is subjective, operator-dependent, shows poor sensitivity for the detection of mild steatosis, and is a poor tool for quantifying the steatosis. Both computed tomography (CT) and magnetic resonance imaging (MRI) seem to be more objective and more sensitive techniques for the quantification of steatosis, but MRI is still less widely available and much more expensive. For the diagnosis of steatosis by CT, the liver-to-spleen attenuation ratio is measured, and the diagnosis of steatosis is made when the ratio is less than 0.9. Of course, CT also has limitations with respect to the diagnosis of steatosis, including poor sensitivity for the detection of mild steatosis, X-ray exposure of the patients, and unavailability for patients with hemosiderosis. Unfortunately, none of these imaging modalities is useful for the diagnosis of NASH.

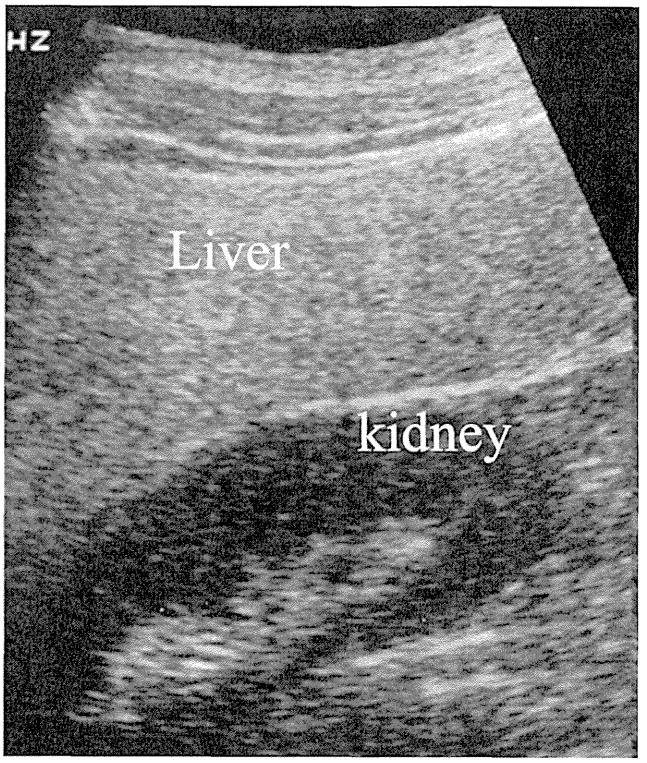


Figure 5 This is an image of ultrasonography. Hepatic steatosis leads to increased hepatorenal contrast, liver brightness, deep attenuation, and vascular blurring. Ultrasonography is an acceptable first-line screening procedure for detection of steatosis in clinical practice.

Concerning interference with the detection of steatosis by advanced fibrosis, the decrease in the detection sensitivity is marked for both US and CT.[34] The sensitivity of US and CT for advanced fibrosis is also decreased markedly in patients with severe steatosis and obese patients, being more marked for US. An awareness of these disadvantages of the common imaging modalities would be useful for a more precise diagnosis of hepatic steatosis and fibrosis in patients with NAFLD.

Liver biopsy. The principal histological features of NASH are as follows: presence of macrovesicular steatosis, ballooning

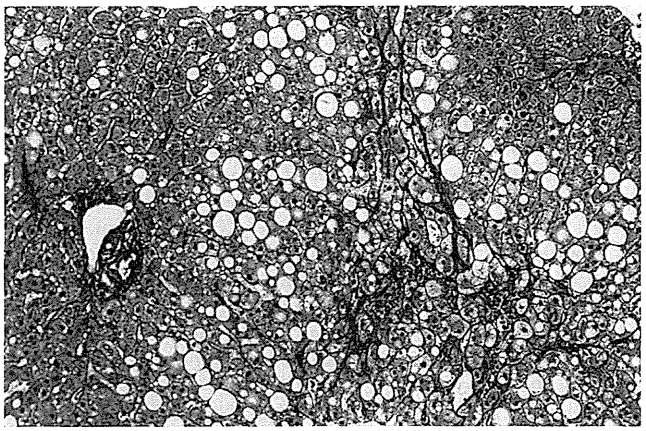


Figure 6 This is a liver biopsy with Mallory staining for fibrosis. Macrovesicular steatosis and prominent pericellular fibrosis around the central vein are present (▼), while portal fibrosis is mild (↓).

degeneration of the hepatocytes, and mixed lobular inflammation. These characteristic pathological features with Mallory hyaline and pericellular fibrosis are predominantly seen around the central veins (zone 3) (Fig. 6). Atypical features have been reported in pediatric cases and morbidly obese cases, such as more periportal steatosis (zone 1), little or no ballooning or Mallory hyaline, and more portal-based chronic inflammation and fibrosis.

Three important pathological classifications have been proposed for NAFLD: Matteoni's classification, Brunt's classification, and the NALFD activity score (NAS).[35–37]

In 1999, Matteoni *et al.*[35] described a classification system that served to distinguish between NASH and non-NASH. They divided 132 NAFLD patients into four categories: type 1, steatosis alone; type 2, steatosis with lobular inflammation only; type 3, steatosis with hepatocellular ballooning; and type 4, type 3 plus either Mallory–Denk bodies or fibrosis. They confirmed the benign clinical course of patients with type 1 or 2 NAFLD and the progressive clinical course of patients who had either type 3 or 4 NAFLD. As a result of these differences, these authors defined type 1 and type 2 histological forms of NAFLD as "non-NASH," and type 3 and type 4 as NASH. However, this classification did not include an assessment of the severity or pattern of NASH, such as the degree of steatosis, inflammation, location of these changes (i.e. lobular or portal), or the degree of fibrosis.

In the same year as Matteoni's classification system was published, Brunt *et al.*[36] proposed a semiquantitative grading and staging system for NASH. This classification was applicable to only NASH and not to the entire spectrum of NAFLD.

In 2005, the NASH Clinical Research Network Pathology Committee developed and validated a histological scoring system based on Brunt's classification, NAS, as a semiquantitative instrument by which to judge treatment responses or disease progression in clinical studies.[37] The NAS system addresses the full spectrum of NAFLD and is applicable to both adult and pediatric NAFLD patients. The score is determined as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3), and ballooning degeneration (0–2). A score of ≥ 5 correlated with the diagnosis of NASH made independently by an experienced pathologist without using the score; likewise, scores of less than 3 were correlated with "not NASH," and scores of 3 or 4 were regarded as borderline. In regard to fibrosis, stage 1 referred to perisinusoidal fibrosis in zone 3 (perivenular area: delicate [1A] and dense [1B]), and detection of portal fibrosis without perisinusoidal fibrosis was defined as 1C. Stage 2 was characterized by perisinusoidal and portal/periportal fibrosis. Stage 3 was defined as bridging fibrosis and stage 4 as cirrhosis. Although the authors reminded us that the NAS system was never intended to be used for the diagnosis of NASH, NAS has frequently been used as a surrogate method for establishing the diagnosis of NASH. Then, they assessed the relation between NASH diagnosed by NAS and pathological diagnosis of steatohepatitis (in this case, NASH) and found that the definitive diagnosis of NASH was not always correlated with threshold values of the NAS.[38] They concluded that clinical pathologists should be encouraged not to use NAS as a categorical approach for the diagnosis of NASH.

Younossi *et al.*[39] assessed the ability to predict the long-term liver-related mortality based on the pathological characteristics. The study cohort consisted of 209 patients with biopsy-proven NAFLD who were followed up for at least 5 years. The results of their multivariate analysis identified only fibrosis as an independent predictor of liver-related mortality. According to the findings of this study, assessment of the severity of hepatic fibrosis is essential for determining the prognosis in patients with NASH.[40]

Indication of liver biopsy. NASH has emerged as a distinct clinicopathological concept, and even now, biopsy evaluation is considered the "gold standard" for a definitive diagnosis. However, liver biopsy has several drawbacks; it is an expensive and invasive procedure and is fraught with the possibility of sampling error and variability in pathologist interpretation. Moreover, given the extremely high prevalence of NAFLD, a liver biopsy would be poorly suited as a diagnostic test for NASH. Accordingly, at present, liver biopsy may only be considered in NAFLD patients who are considered to be at an increased risk of developing NASH with advancing fibrosis or are suspected to have coexisting other chronic liver diseases.[5,6] In general practice, NAFLD is a convenient-to-use term for the diagnosis and management of these patients, and serum biomarkers that indicate the severity of fibrosis serve as clinically useful tools for the identification of NAFLD in patients with bridging fibrosis or cirrhosis.

Non-invasive assessment of NASH and advanced fibrosis in NAFLD. Recently, several biochemical markers and imaging modalities have been reported for predicting NASH and the severity of hepatic fibrosis.[41–47] An ideal biomarker should be simple to measure, accurate, reproducible, inexpensive, and readily available. In general, while most of the biomakers and scoring systems show similar accuracy for the detection of advanced fibrosis, their accuracy is weak for the diagnosis of mild fibrosis. The NAFLD Fibrosis Score is a widely validated scoring system for predicting the severity of fibrosis that is based on six readily assessable clinical variables (age, BMI, hyperglycemia, platelet count, albumin, AST/alanine aminotransferase ratio).[46]

Several imaging techniques have also been advocated as noninvasive diagnostic tests for NASH. US-based transient elastography or FibroScan has shown promising results for assessment of

the severity of liver fibrosis and degree of steatosis. However, these modalities are expensive and not widely available.

Pathogenesis of NASH

The development of NASH is thought to initiate from basal steatosis as the first hit, followed by a "second hit" that is capable of inducing necroinflammation; this hypothesis is the so-called "two-hit theory."[48,49] The second hit can include oxidative stress, especially that arising from mitochondrial stress, insulin resistance, inflammatory cytokines, etc. Autophagy may also play an important role in the pathogenesis of NASH. Recently, a new concept to explain the pathogenesis of NASH was reported by Tilg and Moschen, namely, the "multi-parallel hit" hypothesis.[50] This hypothesis, based on reports that endoplasmic reticulum stress and cytokine-mediated stress can induce steatosis as well as necroinflammation, suggests that multiple hits act together in the development of NASH. Steatosis should therefore be considered as a part of the liver's early "adaptive" response to stress rather than as the first hit in disease progression.

I have summarized the characteristics and diagnosis of NAFLD/NASH. There is still no clear consensus regarding the threshold alcohol consumption for defining "non-alcoholic" liver disease. In the future, a change in the nomenclature of NAFLD/NASH might be needed because there are so many obese people who drink much alcohol and show the histological features of steatohepatitis. Liver biopsy currently remains the gold standard for the diagnosis of NASH. In the future, improved understanding of the pathogenesis of NASH and new technologies may contribute to the diagnostic process and provide reliable non-invasive alternatives to liver biopsy.

References

1 Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin. Proc.* 1980; **55**: 434–8.
2 Sanyal AJ; American Gastroenterogical Association. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**: 1705–25.
3 Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. *Hepatology* 2003; **37**: 1202–19.
4 Farrell GC, Chitturi S, Lau GK *et al.* Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: executive summary. *J. Gastroenterol. Hepatol.* 2007; **22**: 775–7.
5 Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J. Hepatol.* 2010; **53**: 372–84.
6 Chalasani N, Younossi Z, Lavine JE *et al.* The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005–23.
7 Kojima S, Watanabe N, Numata M, Ogawa T, Matsuzaki S. Increase in the prevalence of fatty liver in Japan over the past 12 years: analysis of clinical background. *J. Gastroenterol.* 2003; **38**: 954–61.
8 Amarapurkar DN, Hashimoto E, Lesmana LA *et al.* How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences? *J. Gastroenterol. Hepatol.* 2007; **22**: 788–93.
9 Eguchi Y, Hyogo H, Ono M *et al.* Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study. *J. Gastroenterol.* 2012; **47**: 586–95.
10 Yatsuji S, Hashimoto E, Tobari M, Tokushige K, Shiratori K. Influence of age and gender in Japanese patients with non-alcoholic steatohepatitis. *Hepatol. Res.* 2007; **37**: 1034–43.
11 Hashimoto E, Tokushige K. Prevalence, gender, ethnic variations, and prognosis of NASH. *J. Gastroenterol.* 2011; **46** (Suppl. 1): 63–9.
12 Browning JD, Szczepaniak LS, Dobbins R *et al.* Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387–95.
13 Romeo S, Kozlitina J, Xing C *et al.* Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat. Genet.* 2008; **40**: 1461–5.
14 Kawaguchi T, Sumida Y, Umemura A *et al.* Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. *PLoS ONE* 2012; **7**: e38322.
15 Tokushige K, Takakura M, Tsuchiya-Matsushita N, Taniai M, Hashimoto E, Shiratori K. Influence of TNF gene polymorphisms in Japanese patients with NASH and simple steatosis. *J. Hepatol.* 2007; **46**: 1104–10.
16 Targher G, Bertolini L, Padovani R *et al.* Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2007; **30**: 1212–18.
17 Adams LA, Lymp JF, St Sauver J *et al.* The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113–21.
18 Ekstedt M, Franzén LE, Mathiesen UL *et al.* Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865–73.
19 Rafiq N, Bai C, Fang Y *et al.* Long-term follow-up of patients with nonalcoholic fatty liver. *Clin. Gastroenterol. Hepatol.* 2009; **7**: 234–8.
20 Söderberg C, Stål P, Askling J *et al.* Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology* 2010; **51**: 595–602.
21 Sørensen HT, Mellemkjaer L, Jepsen P *et al.* Risk of cancer in patients hospitalized with fatty liver: a Danish cohort study. *J. Clin. Gastroenterol.* 2003; **36**: 356–9.
22 Teli MR, Day CP, Burt AD, Bennett MK, James OF. Determinants of progression to cirrhosis or fibrosis in pure alcoholic fatty liver. *Lancet* 1995; **346**: 987–90.
23 Dam-Larsen S, Becker U, Franzmann MB, Larsen K, Christoffersen P, Bendtsen F. Final results of a long-term, clinical follow-up in fatty liver patients. *Scand. J. Gastroenterol.* 2009; **44**: 1236–43.
24 Evans CD, Oien KA, MacSween RN, Mills PR. Non-alcoholic steatohepatitis: a common cause of progressive chronic liver injury? *J. Clin. Pathol.* 2002; **55**: 689–92.
25 Hui JM, Kench JG, Chitturi S *et al.* Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C. *Hepatology* 2003; **38**: 420–7.
26 Sanyal AJ, Banas C, Sargeant C *et al.* Similarities and differences in outcomes of cirrhosis due to nonalcoholic steatohepatitis and hepatitis C. *Hepatology* 2006; **43**: 682–9.
27 Yatsuji S, Hashimoto E, Tobari M, Taniai M, Tokushige K, Shiratori K. Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. *J. Gastroenterol. Hepatol.* 2009; **24**: 248–54.
28 Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 1972–8.

29 Adams LA, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J. Hepatol.* 2005; **42**: 132–8.
30 Argo CK, Northup PG, Al-Osaimi AM, Caldwell SH. Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. *J. Hepatol.* 2009; **51**: 371–9.
31 Hashimoto E, Yatsuji S, Tobari M *et al.* Hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *J. Gastroenterol.* 2009; **44** (Suppl. 19): 89–95.
32 Tokushige K, Hashimoto E, Yatsuji S *et al.* Prospective study of hepatocellular carcinoma in nonalcoholic steatohepatitis in comparison with hepatocellular carcinoma caused by chronic hepatitis C. *J. Gastroenterol.* 2010; **45**: 960–7.
33 Yasui K, Hashimoto E, Komorizono Y *et al.* Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma. *Clin. Gastroenterol. Hepatol.* 2011; **9**: 428–33; quiz e50.
34 Tobari M, Hashimoto E, Yatsuji S, Torii N, Shiratori K. Imaging of nonalcoholic steatohepatitis: advantages and pitfalls of ultrasonography and computed tomography. *Intern. Med.* 2009; **48**: 739–46.
35 Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413–19.
36 Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am. J. Gastroenterol.* 1999; **94**: 2467–74.
37 Kleiner DE, Brunt EM, Van Natta M *et al.* Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313–21.
38 Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA; (CRN) NCRN. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. *Hepatology* 2011; **53**: 810–20.
39 Younossi ZM, Stepanova M, Rafiq N *et al.* Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. *Hepatology* 2011; **53**: 1874–82.
40 Angulo P. Diagnosing steatohepatitis and predicting liver-related mortality in patients with NAFLD: two distinct concepts. *Hepatology* 2011; **53**: 1792–4.
41 Hashimoto E, Farrell GC. Will non-invasive markers replace liver biopsy for diagnosing and staging fibrosis in non-alcoholic steatohepatitis? *J. Gastroenterol. Hepatol.* 2009; **24**: 501–3.
42 Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann. Med.* 2011; **43**: 617–49.
43 Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. *Hepatology* 2009; **50**: 1072–8.
44 Sumida Y, Yoneda M, Hyogo H *et al.* Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD). A simple clinical scoring system using ferritin, fasting insulin, and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease. *J. Gastroenterol.* 2011; **46**: 257–68.
45 Kaneda H, Hashimoto E, Yatsuji S, Tokushige K, Shiratori K. Hyaluronic acid levels can predict severe fibrosis and platelet counts can predict cirrhosis in patients with nonalcoholic fatty liver disease. *J. Gastroenterol. Hepatol.* 2006; **21**: 1459–65.
46 Angulo P, Hui JM, Marchesini G *et al.* The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846–54.
47 Guha IN, Parkes J, Roderick P *et al.* Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology* 2008; **47**: 455–60.
48 Day CP, James OF. Steatohepatitis: a tale of two "hits." *Gastroenterology* 1998; **114**: 842–5.
49 Stärkel P, Sempoux C, Leclercq I *et al.* Oxidative stress, KLF6 and transforming growth factor-beta up-regulation differentiate non-alcoholic steatohepatitis progressing to fibrosis from uncomplicated steatosis in rats. *J. Hepatol.* 2003; **39**: 538–46.
50 Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* 2010; **52**: 1836–46.

Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/jgh.12239

NUTRITION-RELATED LIVER DISORDERS: NAFLD

Hepatocarcinogenesis in non-alcoholic fatty liver disease in Japan

Katsutoshi Tokushige, Etsuko Hashimoto and Kazuhiko Kodama

Department of Internal Medicine and Gastroenterology, Tokyo Women's Medical University, Tokyo, Japan

Key words
hepatocellular carcinoma (HCC), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH).

Accepted for publication 4 March 2013.

Correspondence
Dr Katsutoshi Tokushige, 8-1 Kawada-cho Shinjuku-ku, Tokyo 162-8666, Japan. Email: ktoku@pg7.so-net.ne.jp

Abstract

In Japan, there has been a gradual increase in cases of non-viral chronic liver diseases, including non-alcoholic fatty liver disease (NAFLD), occurring with hepatocellular carcinoma (HCC). First, a national survey investigating the etiology of HCC in Japan was performed. Among HCCs based on non-viral disease, alcoholic liver disease with HCC accounted for 7.2% of all HCCs, followed by chronic liver disease of unknown etiology with HCC (5.1%) and NAFLD with HCC (2.0%). The clinical characteristics of these three HCC groups were clearly different. In our second analysis, the HCC development rates among liver cirrhosis with NAFLD, alcoholic cirrhosis, and cirrhosis with hepatitis C virus (HCV) were compared. HCC development rates were 11.3%/5 years in NAFLD cirrhosis, 30.5%/5 years in HCV cirrhosis, and 12.5%/5 years in alcoholic cirrhosis, suggesting that the hepatocarcinogenesis in NAFLD and alcoholic liver disease were similar but were lower than that in HCV.

Using Cox hazards analysis, older age, higher serum γ-glutamyl transpeptidase level, and higher Child–Pugh score as risk factors of HCC were identified. Finally, clinical data of NAFLD-HCC with the data for HCC with HCV (HCV-HCC) were compared. The percentage of NAFLD-HCC patients with des-gamma-carboxy prothrombin-positive was higher than that with α-fetoprotein-positive. The 5-year survival and recurrence rates for NAFLD-HCC were almost similar to those for HCV-HCC. In Asian countries, the prevalence of NAFLD is increasing. Therefore, elucidating the pathogenesis and clinical features of HCC in patients with NAFLD is indeed an urgent problem.

Introduction

Primary liver cancer is the fifth most common cancer worldwide and the third most common cause of cancer mortality.[1,2] Hepatocellular carcinoma (HCC) accounts for about 90% of primary liver cancers. With respect to the underlying liver disease, the latest nationwide report of the Liver Cancer Study Group of Japan showed that hepatitis C virus (HCV)-related liver disease is the most common underlying cause of HCC.[3] HCV-related HCC accounts for 67% of all HCC cases, followed by 16% for hepatitis B virus (HBV)-related HCC. The incidence of HCV-related HCC has been gradually decreasing in recent years, while the incidence of HCC associated with non-viral chronic liver disease has gradually been increasing.

In Pacific and Asian countries, the prevalence of non-alcoholic fatty liver disease (NAFLD) in the general population is increasing dramatically and ranges from 5% to 40%.[4,5] NAFLD consists of simple steatosis and non-alcoholic steatohepatitis (NASH), while NASH comprises a wide spectrum of conditions from NASH without fibrosis to cirrhosis. Obesity and diabetes mellitus have been established as significant risk factors for HCC by epidemiological observations and experimental studies,[6,7] and there is increasing evidence that NASH is a risk factor for HCC.[8,9] We reported that HCC was a critical factor in the prognosis of NAFLD cirrhosis.[10] Therefore, there is an urgent need to elucidate pathogenesis, clinical features, and treatments for these diseases, especially NAFLD advanced stages and NAFLD-related HCC (NAFLD-HCC).

In this review, we describe the survey of HCC in Japan that my colleagues and I conducted, the rate at which HCC develops from NAFLD, the risk factors for HCC in NAFLD, the clinical features of NAFLD-HCC.

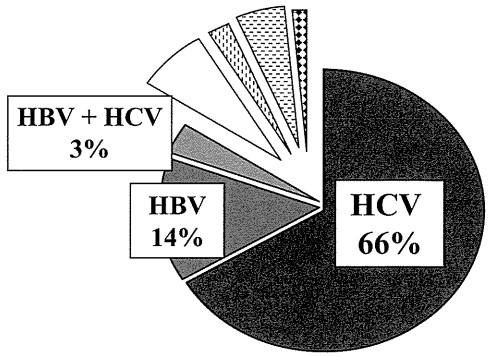
National survey of HCC

We performed a national survey investigating the etiology of HCC in the Japanese population in 2010. The nationwide survey included 14 530 HCC patients diagnosed during 2006–2009,[11] of whom 14.1% were positive for hepatitis B surface (HBs) antigen, 66.3% were positive for HCV-RNA, and 3.7% were positive for both HBs antigen and HCV-RNA. Among those surveyed, 15.8% of patients were diagnosed as having non-HBV, non-HCV HCC.

Among HCCs based on non-viral disease, alcoholic liver disease with HCC (ALD-HCC) accounted for 7.2% of all HCCs, followed by chronic liver disease of unknown etiology with HCC (unknown HCC) (5.1%) and NAFLD with HCC (2.0%) (Fig. 1). The characteristics of these three groups were clearly different from one another (median age was 72 years for NAFLD-HCC, 68 years for ALD-HCC, and 73 years for unknown HCC, $P < 0.01$; female gender was 38%, 4%, and 37%, respectively, $P < 0.01$) (Table 1). Body mass index (BMI) and the prevalence of diabetes, hypertension, and dyslipidemia were significantly higher in patients with NAFLD-HCC than in those with ALD-HCC and unknown HCC. Serum levels of total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (GTP) were significantly higher in the ALD-HCC group compared with the other groups, while the platelet count and serum albumin level were lowest in the ALD-HCC group. The hemoglobin A_{1C} and fasting blood glucose levels were highest in the NAFLD-HCC group. These data suggested that clinical characteristics of these three HCC groups were clearly different from one another.

Regarding the etiology of HCC in Western countries, NAFLD-HCC has been reported to account for 3.8–13% of all HCCs.[12,13] In comparison with Western countries, the prevalence of NAFLD-HCC is lower in Japan. This is not only due to the low incidence of NAFLD-HCC but also to the high incidence of hepatitis virus-related HCC in Japan. However, the incidence of NAFLD-HCC in Japan is expected to increase in the future because of the rising prevalence of NAFLD associated with obesity and/or diabetes.

To determine whether modest alcohol intake could influence carcinogenesis in patients with unknown HCC, we divided the patients into a no alcohol subgroup (alcohol consumption < 20 g/day) and a modest alcohol intake subgroup (alcohol consumption of 20–70 g/day) (Table 2). Among the no alcohol subgroup, the prevalence of women was markedly higher ($P < 0.001$) at 58%



Alcoholic -HCC	**7.2%**
Etiology unknown-HCC	**5.1%**
NAFLD-HCC	**2.0%**
Autoimmune Liver diseases (PBC + AIH + PSC)*	**1.2%**
Congestive disease	**0.1%**
Metabolic disease**	**0.1%**

Figure 1 National survey in Japan. (2006–2009). Among the 14 530 patients with hepatocellular carcinoma (HCC), 14.1% were positive for hepatitis B surface (HBs) antigen, 66.3% were positive for hepatitis C virus (HCV)-RNA, and 3.7% were positive for both HBs antigen and HCV-RNA. Among the HCC patients with non-viral liver diseases, alcoholic liver disease with HCC (ALD-HCC) (7.2%) was the most common diagnosis, followed by unknown HCC (5.1%). Non-alcoholic fatty liver disease (NAFLD)-HCC (2.0%) was the third most common etiology. (■) HCV; (■) hepatitis B virus (HBV); (■) HBV + HCV; (□) alcoholic; (⊡) NAFLD; (⊟) etiology unknown; (⊞) others. *AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis. **Metabolic disease; Wilson disease, hemochromatosis, etc. Adapted from Tokushige *et al.*[11]

Table 1 Comparison among NAFLD-HCC, ALD-HCC, and unknown HCC

	NAFLD-HCC ($n = 292$)	ALD-HCC ($n = 991$)	Unknown-HCC ($n = 614$)	*P* value
Age at diagnosis	72 ± 8.4	68 ± 9.1	73 ± 10.1	< 0.001
Gender (female)	38%	4%	37%	< 0.001
BMI(kg/m^2)	27.0 ± 4.0	23.8 ± 3.7	23.5 ± 4.1	< 0.001
Diabetes	70%	49%	43%	< 0.001
Hypertension	60%	43%	46%	< 0.001
Dyslipidemia	35%	14%	15%	< 0.001
Liver cirrhosis	62%	78%	52%	< 0.001
Albumin (g/dL)	3.8 ± 0.6	3.6 ± 0.6	3.6 ± 0.6	< 0.001
Total bilirubin (mg/dL)	0.9 ± 1.3	1.1 ± 1.9	0.9 ± 1.7	< 0.001
AST (IU/L)	40 ± 36	80 ± 301	43 ± 71	< 0.001
ALT (IU/L)	35 ± 35	45 ± 176	30 ± 44	0.03
γ-GTP (IU/L)	91 ± 202	147 ± 271	88 ± 198	< 0.001
FBS (mg/dL)	119 ± 57	111 ± 63	107 ± 53	< 0.001
HbA_{1c} (%)	6.3 ± 1.4	5.9 ± 1.6	5.7 ± 1.4	< 0.001
Platelet count (× 10^4/mm^3)	14.1 ± 7.4	12.6 ± 8.0	15.2 ± 9.1	< 0.001
AFP (ng/mL)	12 ± 427 557	11 ± 368 512	13.0 ± 94 155	0.284

Adapted from Tokushige *et al.*[11]

γ-GTP, gamma-glutamyl transpeptidase; AFP, α-fetoprotein; ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, fasting blood sugar; HbA_{1C}, hemoglobin A_{1C}; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease.

Table 2 Comparison between the no alcohol and modest alcohol subgroups of unknown HCC

	No alcohol intake $n = 316$	Modest alcohol intake $n = 214$	*P* value
Age at diagnosis	75.5 ± 10.2	72 ± 9.0	< 0.001
Gender (female)	58%	8%	< 0.001
BMI (kg/m^2)	23.8 ± 4.5	23.5 ± 3.4	0.396
Diabetes	41%	46%	0.214
Hypertension	45%	49%	0.424
Dyslipidemia	15%	15%	0.989
Liver cirrhosis	57%	42%	0.001
Albumin (g/dL)	3.6 ± 0.7	3.8 ± 0.6	0.030
Total bilirubin (mg/dL)	0.9 ± 1.5	0.8 ± 1.2	0.266
AST (IU/L)	44 ± 63	39 ± 73	0.081
ALT (IU/L)	29 ± 45	29 ± 42	0.455
γ-GTP (IU/L)	75 ± 184	103.5 ± 213	0.003
FBS (mg/dL)	106 ± 51	110 ± 56	0.050
HbA_{1c} (%)	5.7 ± 1.3	5.7 ± 1.5	0.307
Platelet count (× 10^4/mm^3)	14.6 ± 9.0	16.8 ± 8.7	0.001
AFP (ng/mL)	13.3 ± 77 396	10 ± 31 196	0.378

Adapted from Tokushige *et al.*[11]

γ-GTP, gamma-glutamyl transpeptidase; AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, fasting blood sugar; HbA_{1c}, hemoglobin A_{1c}; HCC, hepatocellular carcinoma.

versus only 8% in the modest alcohol subgroup. The mean age at diagnosis of HCC was higher in the no alcohol subgroup than in the modest alcohol intake subgroup (75.5 years *vs* 72 years, $P < 0.001$). Between the two subgroups, the modest alcohol intake subgroup showed different clinical features in terms of unknown HCC and showed the same trends in regard to gender, BMI, lifestyle-related diseases, and γ-GTP levels as the ALD-HCC group.

These data suggested that a relatively low alcohol intake may lead to the development of non-viral HCC. The alcohol consumption criteria for diagnosis of alcoholic liver disease vary around the world,[14,15] and the alcohol consumption criterion for alcoholic liver disease proposed by the Japanese Study Group of Alcoholic Liver Disease is more than 70 g/day. Our data suggest that social or modest intake of alcohol might have a more significant role in hepatic carcinogenesis than is presently thought. In the future, more detailed studies will need to be performed, including assessment of alcohol metabolism genotypes.

HCC rate in patients with NAFLD

Kawamura *et al.* reported that rate of HCC was 0.51%/12 years from all NAFLD, including simple steatosis.[16] In NAFLD as a whole, the development of HCC is rare. However, liver fibrosis is the most important factor for development of HCC in any liver disease. To make clear the hepatocarcinogenic power in NAFLD, we compared the HCC development rates among liver cirrhosis (LC) with NAFLD (NAFLD-LC), alcoholic cirrhosis (ALD-LC), and cirrhosis infected with HCV (HCV-LC) in our hospital. HCC development rates were 11.3%/5 years in NAFLD-LC, 30.5%/5 years in HCV-LC, and 12.5%/5 years in ALD-LC (Fig. 2).[10,17] Sanyal *et al.* and Ascha *et al.* reported that the HCC development

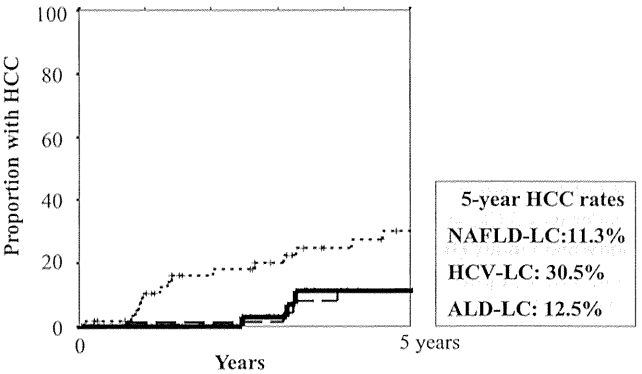


Figure 2 Hepatocellular carcinoma (HCC) rate in non-alcoholic fatty liver disease (NAFLD) cirrhosis (NAFLD-LC), alcoholic liver disease-liver cirrhosis (ALD-LC), and hepatitis C virus (HCV)-liver cirrhosis (HCV-LC). The HCC rates in NAFLD-LC and ALD-LC were similar, and were lower than that in HCV-LC. (▬) NAFLD; (— –) ALD; (·····) HCV. Adapted from Yatsuji *et al.* and Kodama *et al.*[10,17]

Table 3 The comparison between NAFLD-HCC ($n = 41$) and NAFLD without HCC ($n = 533$) by multivariate logistic regression model

	Odds ratio	95% CI	*P* value
Age (older)	1.103	1.050~1.159	< 0.001
Gender (male)	4.680	1.803~12.146	0.002
Liver fibrosis	2.718	1.745~4.233	< 0.001
Activity	0.361	0.163~0.802	0.012
ALT	0.974	0.955~0.993	0.007
γ-GTP	1.005	1.001~1.009	0.008

γ-GTP, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; CI, confidence interval; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease.

rate in NAFLD cirrhosis was about 10–13% in 5 years and lower than that of HCV-LC in the USA.[18,19] These data almost match the Japanese data. The rates of hepatocarcinogenesis in NAFLD and alcoholic liver disease were almost identical but were lower than that in chronic HCV liver disease.

Risk factors of NAFLD-HCC

To clarify the risk factors of HCC in NAFLD, we compared clinical data between NAFLD-HCC and NAFLD without HCC with a multivariate logistic regression model. Both NAFLD patients with and without HCC were admitted to our hospital between 1990 and 2011. NAFLD was diagnosed by liver biopsy. Age, gender, BMI, diabetes, hypertension, dyslipidemia, blood examinations (total bilirubin, albumin, AST, ALT, alkaline phosphatase [ALP], γ–GTP, platelet, prothrombin time [PT]), and liver histology findings (fibrosis grade, activity grade, and steatosis grade) were analyzed as risk factors of HCC. In the results, older age, male gender, advanced liver fibrosis, lower activity of liver histology, lower ALT level, and higher γ-GTP level were detected as risk factors of HCC in the population with NAFLD-HCC (Table 3). However, this analysis did not include the factor of duration, and liver fibrosis is the most important factor for

Table 4 Risk factors for HCC in the NAFLD-LC(n = 72) according to the Cox hazards model

	Hazard ratio	95% CI	P value
Age (older)	1.12	1.014–1.226	0.024
γ-GTP	1.01	1.002–1.022	0.023
Child–Pugh score	3.09	1.374–6.934	0.006

Adapted from Kodama *et al.*[17]
γ-GTP, gamma-glutamyl transpeptidase; CI, confidence interval; HCC, hepatocellular carcinoma; LC, liver cirrhosis; NAFLD, non-alcoholic fatty liver disease.

development of HCC. In the next analysis, we investigated the risk factors for HCC in 72 NAFLD-LC patients with a Cox hazards model. All NAFLD-LC patients were admitted to our hospital between 1990 and 2011. NAFLD-LC was diagnosed by liver biopsy. The patients with NAFLDH-LC were assessed with regard to the development of HCC, and their risk factors for HCC were analyzed. Age, gender, BMI, ascites, varices, encephalopathy, diabetes, hypertension, dyslipidemia, blood examinations (total bilirubin, albumin, AST, ALT, ALP, hypertension, γ-GTP, platelet, PT), and Child–Pugh score were analyzed as risk factors of HCC. Older age, higher serum γ-GTP level, and higher Child–Pugh score were identified as risk factors in NAFLD-LC (Table 4), and older age and Child–Pugh were confirmed by log-rank test.[17] Kawamura *et al.* reported the risk factors for HCC in all NAFLD patients as being old age, AST > 40 IU/mL, advanced fibrosis, and diabetes mellitus.[16] Ascha *et al.* reported that NASH patients with cirrhosis had a greatly increased risk of liver cancer, and even social alcohol consumption appeared to be the most significant factor associated with the risk of HCC.[19] Considering all of these findings, we conclude that older age, male gender, advanced fibrosis, γ-GTP level, which was the marker of oxidative stress, diabetes mellitus, and mild alcohol intake might be important factors in the pathogenesis of HCC in NAFLD.

Clinical features of NAFLD-HCC

Finally, we compared the clinical data of NAFLD-HCC with the data for HCC caused by HCV infection (HCV-HCC) in our hospital. The percentage of NAFLD-HCC patients with des-gamma-carboxy prothrombin-positive results was higher than that of patients with α-fetoprotein-positive results.[20] Yasui *et al.* also showed the same profile of tumor markers in NASH-HCC.[21] In our hospital, the 5-year survival rate in the treated NAFLD-HCC group was 55.2%, and the cumulative HCC recurrence rate at 5 years was 69.8% as opposed to a 5-year survival rate of 50.6% and recurrence rate of 83.1% in the HCV-HCC group.[22] The 5-year survival and recurrence rates for NAFLD-HCC were almost similar to those for HCV-HCC.

Zen *et al.* reported a case of HCC arising in a patient diagnosed with NASH at 62 years old. At 66 years old, her first hepatic tumor appeared. The pathological diagnosis of the first nodule was "pseudolymphoma." When she was 72 years old, three hepatic tumors appeared and were diagnosed as moderately differentiated HCC. At age 73, two more tumors appeared and were diagnosed as well-differentiated HCC and a dyplastic nodule.[23] These results suggested a multicentric occurrence of HCC in NASH, similar to HCC based on viral hepatitis.

We had measured anti-hepatitis B core (HBc) antibody to investigate the influence of HBV on the carcinogenesis of NAFLD-HCC. The difference between the NAFLD-HCC group and HCV-HCC group was not significant, and none of the NAFLD-HCC patients had high HBc antibody titers that would have led to the suspicion that they were HBV carriers. These findings therefore suggested that even if HBV did influence carcinogenesis in NAFLD, the influence would be minimal.

We reported that HCC was a critical factor in the prognosis of NAFLD.[10] Regular screening for HCC is extremely important, especially in NAFLD patients with advanced fibrosis, and the strong possibility of recurrence also warrants close attention.

In conclusion, in Asian countries, the prevalence of NAFLD is increasing dramatically. Elucidating the pathogenesis, clinical features, and treatment of HCC in NAFLD is an urgent problem.

References

1 Bosch FX, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004; **127** (5 Suppl. 1): S5–S16.
2 Sherman M. Hepatocellular carcinoma: epidemiology, risk factors and screening. *Semin. Liver Dis.* 2005; **25**: 143–5.
3 Ikai I, Arii S, Okazaki M *et al.* Report of the 17th nationwide follow-up survey of primary liver cancer in Japan. *Hepatol. Res.* 2007; **37**: 676–91.
4 Chitturi S, Farrell GC, Hashimoto E, Saibara T, Lau GK, Sollano JD, Asia-Pacific Working Party on NAFLD. Non-alcoholic fatty liver disease in the Asia-Pacific region: definitions and overview of proposed guidelines. *J. Gastroenterol. Hepatol.* 2007; **22**: 778–87.
5 Amarapurkar DN, Hashimoto E, Lesmana LA, Sollano JD, Chen PJ, Goh KL, Asia-Pacific Working Party on NAFLD. How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences? *J. Gastroenterol. Hepatol.* 2007; **22**: 788–93.
6 Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology* 2010; **51**: 1820–32.
7 Siegel AB, Zhu AX. Metabolic syndrome and hepatocellular carcinoma: two growing epidemics with a potential link. *Cancer* 2009; **115**: 5651–61.
8 Shimada M, Hashimoto E, Taniai M *et al.* Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. *J. Hepatol.* 2002; **37**: 154–60.
9 Bugianesi E. Non-alcoholic steatohepatitis and cancer. *Clin. Liver Dis.* 2007; **11**: 191–207.
10 Yatsuji S, Hashimoto E, Tobari M, Taniai M, Tokushige K, Shiratori K. Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. *J. Gastroenterol. Hepatol.* 2009; **24**: 248–54.
11 Tokushige K, Hashimoto E, Horie Y, Taniai M, Higuchi S. Hepatocellular carcinoma in Japanese patients with nonalcoholic fatty liver disease, alcoholic liver disease and chronic liver disease of unknown etiology: report of the Nationwide Survey. *J. Gastroenterol.* 2011; **46**: 1230–7.
12 Malik SM, Gupte PA, de Vera ME, Ahmad J. Liver transplantation in patients with nonalcoholic steatohepatitis-related hepatocellular carcinoma. *Clin. Gastroenterol. Hepatol.* 2009; **7**: 800–6.
13 Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. *Hepatology* 2002; **36**: 1349–54.
14 Mandayam S, Jamal MM, Morgan TR. Epidemiology of alcoholic liver disease. *Semin. Liver Dis.* 2004; **24**: 217–32.

15 Bellentani S, Saccoccio G, Costa G *et al.* Drinking habits as cofactors of risk for alcohol induced liver damage. The Dionysos Study Group. *Gut* 1997; **41**: 845–50.
16 Kawamura Y, Arase Y, Ikeda K *et al.* Large-scale long-term follow-up study of Japanese patients with non-alcoholic fatty liver disease for the onset of hepatocellular carcinoma. *Am. J. Gastroenterol.* 2012; **107**: 253–61.
17 Kodama K, Tokushige K, Hashimoto E, Taniai M, Shiratori K. Hepatic and extrahepatic malignancies in cirrhosis caused by nonalcoholic steatohepatitis and alcoholic liver disease. *Alcohol. Clin. Exp. Res.* 2013; **37** (Suppl. 1): E247–52.
18 Sanyal AJ, Banas C, Sargeant C *et al.* Similarities and differences in outcomes of cirrhosis due to nonalcoholic steatohepatitis and hepatitis C. *Hepatology* 2006; **43**: 682–9.
19 Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 1972–8.
20 Hashimoto E, Tokushige K. Hepatocellular carcinoma in non-alcoholic steatohepatitis: growing evidence of an epidemic? *Hepatol. Res.* 2012; **42**: 1–14.
21 Yasui K, Hashimoto E, Komorizono Y *et al.* Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma. *Clin. Gastroenterol. Hepatol.* 2011; **9**: 428–33.
22 Tokushige K, Hashimoto E, Yatsuji S *et al.* Prospective study of hepatocellular carcinoma in nonalcoholic steatohepatitis in comparison with hepatocellular carcinoma caused by chronic hepatitis C. *J. Gastroenterol.* 2010; **45**: 960–7.
23 Zen Y, Katayanagi K, Tsuneyama K, Harada K, Araki I, Nakanuma Y. Hepatocellular carcinoma arising in non-alcoholic steatohepatitis. *Pathol. Int.* 2001; **51**: 127–31.

J Gastroenterol (2014) 49:1352–1361
DOI 10.1007/s00535-013-0899-6

The Japanese Society of Gastroenterology

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Alcohol consumption and recurrence of non-B or non-C hepatocellular carcinoma after hepatectomy: a propensity score analysis

Atsushi Kudo · Shinji Tanaka · Daisuke Ban · Satoshi Matsumura · Takumi Irie · Takanori Ochiai · Noriaki Nakamura · Shigeki Arii · Minoru Tanabe

Received: 29 May 2013 / Accepted: 4 October 2013 / Published online: 18 October 2013

Abstract
Background The aim of this study was to identify factors related to the recurrence of non-B or non-C (NBNC) hepatocellular carcinoma (HCC).
Study design Between April 2000 and March 2012, out of 621 consecutive HCC patients at our institution, 543 who underwent initial hepatectomy and had no extrahepatic metastases were enrolled in the study. Multivariate analysis were performed to identify risk factors for poor disease-free survival (DFS).
Results The 5-year DFS rate of NBNC (34 %) was better than that of hepatitis virus B (30 %, $P = 0.011$) and hepatitis virus C (21 %, $P < 0.0001$), significantly. Multivariate analysis revealed NBNC [hazard ratio (HR), 0.5; 95 % CI, 0.4–0.8; $P < 0.0001$)] to be an independent factor for DFS rate. We constructed a propensity score matching model with the 543 patients, and the 5-year DFS rates with and without severe alcohol liver disease (ALD) were 31.6 and 47.5 %, respectively ($P = 0.013$). In the 163 NBNC patients, severe ALD, mild ALD, and no ALD were seen in 35, 56, and 72 patients, respectively. Multivariate analysis revealed a vascular invasion into the hepatic vein (HR, 3.3; 95 % CI, 1.7–6.3; $P < 0.0001$) and severe ALD (HR, 2.0; 95 % CI, 1.1–3.6; $P = 0.020$) to be independent risk factors for poor DFS. By propensity score matching between mild and severe ALD, the 5-year DFS rates with severe and mild ALD were 26 and 50 %, respectively ($P = 0.035$).
Conclusions The prognoses of NBNC patients were better than those of patients with viral infections. Among the NBNC patients, preoperative excessive alcohol intake decreased DFS rate of HCC occurrence after surgery.

Keywords Hepatitis B virus · Hepatitis C virus · Non-B non-C · Hepatocellular carcinoma · Recurrence · Hepatectomy

Abbreviations

AFP	Alpha-fetoprotein
DCP	Des-gamma-carboxy prothrombin
DFS	Disease-free survival
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
NBNC	Non-B non-C
HR	Hazard ratio
OS	Overall survival

Introduction

Primary liver cancer involving hepatocellular carcinoma (HCC) is the fifth most common and fatal cancer worldwide. HCC has been the most rapidly increasing cancer-related cause of death in developed countries including Japan, Australia, Canada, the United States, and throughout Europe over the last two decades. The number of non-B non-C (NBNC) HCC patients has increased rapidly [1]. Chronic viral hepatitis and liver cirrhosis following

Electronic supplementary material The online version of this article (doi:10.1007/s00535-013-0899-6) contains supplementary material, which is available to authorized users.

A. Kudo (✉) · S. Tanaka · D. Ban · S. Matsumura · T. Irie · T. Ochiai · N. Nakamura · S. Arii · M. Tanabe
Department of Hepatobiliary-Pancreatic Surgery, Graduate School of Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
e-mail: kudomsrg@tmd.ac.jp

hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are responsible for most HCCs. The oncogenic mechanism and clinicopathological characteristics of HCCs critically depend on the type of hepatitis virus involved [2, 3]. Patients with HBV-related HCCs may have a better liver function reserve than those with HCV-related tumors. The etiology is unclear in the other 15–50 % of new HCC cases. In Japan, 10 % of patients diagnosed with HCC have NBNC HCC.

Most patients with NBNC HCC have alcoholic liver disease or nonalcoholic fatty liver disease (NAFLD) including nonalcoholic steatohepatitis (NASH). Recent studies have indicated that both NASH and excessive alcohol intake increase the risk of developing HCC [4]. The prevalence of NAFLD and NASH is reported to be 20 %and 1 %, respectively, among adults in Japan [5, 6], and longitudinal outcome studies have reported that the prevalence of HCC in patients with NAFLD and NASH is 0–0.5 and 0–2.8 %, respectively, over a period of up to 19.5 years [7–10]. In Europe, alcohol-induced cirrhosis accounts for one-third to one-half of all HCC cases [11–13]. HCC is found in 10.1 % of patients with cirrhosis caused by alcohol alone, and its prevalence is almost identical to that of HCV infection [14]. The risk of developing HCC increases when daily alcohol consumption exceeds 80 g/day, whereas the adjusted odds ratio is not increased significantly for patients who consume alcohol at less than 80 g/day [15]. However, some reports indicate that patients with NBNC HCC present with more advanced tumors with poor differentiation, invasion, and vascular involvement and a higher incidence of intrahepatic metastases than patients with HCCs associated only with viral infection [16–18].

In this study, we aimed to elucidate the clinicopathological features of patients with NBNC HCC who had undergone hepatectomy, and the factors, including preoperative alcoholism, that are associated with recurrence. For a fair comparison, key factors that were responsible for DFS were adjusted for by using propensity score-matched analysis. Moreover, we examined whether alcoholism promotes the recurrence of HCC after hepatectomy and whether preoperative alcohol consumption is the best predictor of DFS in patients with NBNC HCC.

Patients and methods

Between April 2000 and March 2012, a total of 621 patients received initial treatment for HCC at the Department of Hepatobiliary Pancreatic Surgery, Tokyo Medical and Dental University. Of these patients, 545 patients underwent initial hepatectomy for HCC and were not found to have extrahepatic metastases. The 543 patients, excluding two (one with autoimmune hepatitis and one with primary biliary cirrhosis), were enrolled in the unadjusted study. The baseline characteristics of the patients are shown in Table 1 (The data of four HBV + HCV patients are not shown). We classified NBNC patients into severe ALD group (alcohol consumption $\geq$ 80 g/day), no ALD (alcohol consumption $<$ 20 g/day), and mild ALD group (20 g/day $\leq$ alcohol consumption $<$ 80 g/day). Alcoholic history was available in 463 patients. Occult HBV infection is defined by the absence of serologically detectable HBs antigen despite the presence of HBc antibody in serum [19, 20].

The decision to perform hepatic resection with anatomical resection is generally determined by the Child-Pugh A/B score and the indocyanine green retention rate at 15 min (ICG-R15) according to the Makuuchi criteria. Non-anatomic resection includes partial resection. In the anatomic resections performed in our study, the liver was divided along the demarcation line after occlusion of the portal vein and hepatic artery. When necessary, the main feeding artery was identified by intravenous injection of sonazoid [21]. We divided the liver parenchyma using an ultrasonic dissector and other energy devices. Prior to resection, all tumors were examined by intraoperative ultrasonography and preoperative computed tomography (CT). Intraoperative ultrasonography with contrast enhancement was used, if necessary [22]. The size of the tumors and length of the surgical margin were measured before fixation of the specimens. The extent of macrovascular invasion was determined using preoperative CT, as microvascular invasion could not be determined before hepatectomy. Microvascular invasion was evaluated on the basis of histological findings if macrovascular invasion was not noted. Background liver cirrhosis and surgical margins were assessed by microscopic examination of the specimens. After discharge, all the patients were examined for recurrence by ultrasonography every 3 months and by dynamic CT every 6 months. The median follow-up period after surgery was 2.9 years (range 0–11.2 years). DFS was defined as the interval between the operation and the date on which recurrence was diagnosed or the end of the observation period if no recurrence was noted. The general rules for the clinical and pathological study of primary liver cancer by liver cancer study group of Japan (5th edition, revised version) simply classify the liver histology into normal liver, chronic hepatitis, and liver cirrhosis. The rule describes the classification of the hepatic fibrosis in detail, as follows: no fibrosis (f0), increased fibrosis of portal area (f1), bridging fibrosis (f2), bridging fibrosis with distorted hepatic lobules (f3), and liver cirrhosis (f4). The patients' medical records were reviewed systematically for relevant clinical data (gender, age, viral infection, alcohol use, and liver function), tumor factors (primary tumor size and

Table 1 Baseline characteristics of patients with non-B non-C hepatocellular carcinoma

	HBV ($N = 96$)	HCV ($N = 275$)	NBNC ($N = 168$)	P
Age (years)	59.3 ± 11.4	68.4 ± 7.6	68.5 ± 11.2	<0.0001*
Gender				
Male	74 (77 %)	200 (73 %)	141 (84 %)	0.025*
Alcoholism (+)	21 (25 %)	66 (26 %)	100 (60 %)	<0.0001*
Severe ALD (+)	9 (12 %)	18 (8 %)	35 (24 %)	<0.0001*
Liver function				
ICG-R15 (%)	15.1 ± 11.7	19.3 ± 11.4	15.2 ± 9.5	<0.0001*
AST (IU/L)	48.7 ± 45.8	60.3 ± 41.7	42.6 ± 27.0	<0.0001*
Platelet (10^4/mL)	16.2 ± 8.1	13.5 ± 6.7	19.5 ± 11.0	<0.0001*
Prothrombin time (%)	84.5 ± 18.2	85.9 ± 15.3	86.1 ± 16.6	0.853
Albumin (g/dL)	4.0 ± 0.5	3.8 ± 0.6	4.0 ± 0.4	<0.0001*
Total bilirubin (mg/dL)	1.0 ± 1.0	0.8 ± 0.4	0.9 ± 0.5	0.443
Child Pugh score	4.9 ± 1.6	5.2 ± 1.3	4.8 ± 1.7	0.092
Tumor factors				
Tumor size (cm)	5.4 ± 4.4	4.0 ± 2.5	5.8 ± 4.1	<0.0001*
Tumor number	1.5 ± 1.1	1.6 ± 1.0	1.5 ± 1.2	0.98
Alpha-fetoprotein (ng/mL)	12854 ± 66264	3497 ± 27261	2477 ± 14500	0.179
DCP (AU/L)	6267 ± 31637	3351 ± 17435	11644 ± 44165	0.101
Anatomic resection (+)	68 (71 %)	162 (59 %)	123 (73 %)	0.017*
Pathological findings				
Micro-vascular invasion				
vp (+)	49 (51 %)	101 (37 %)	69 (41 %)	0.102
vv (+)	12 (13 %)	33 (12 %)	22 (15 %)	0.829
b (+)	5 (5 %)	13 (5 %)	15 (9 %)	0.188
Chronic hepatitis (+)	35 (36 %)	112 (41 %)	65 (40 %)	0.793
Liver cirrhosis (+)	50 (52 %)	153 (56 %)	55 (34 %)	<0.0001*
Surgical margin (+)	19 (20 %)	53 (19 %)	23 (14 %)	0.270

Values are shown as the mean ± SD

ALD alcoholic disease, *DCP* des-gamma-carboxy prothrombin, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *NBNC* non-HBV non-HCV

* $P < 0.05$ considered statistically significant

tumor markers), operative procedure, and pathological findings. We determined alcoholism as some mental and/or physical status related to alcohol dependence [23]. Follow-up data were updated yearly or at shorter intervals, and the last follow-up examination was performed in March 2012.

Statistical analysis were performed using SPSS version 21.0 (IBM Inc., Chicago, IL, USA), unless otherwise stated. Analysis of variance and the χ^2 test were used for continuous and categorical data, respectively. The odds ratio for recurrence for each factor was examined by univariate analysis using the Cox proportional hazards model. Variables found to be statistically significant on this basis were entered into multivariate analysis. DFS was analyzed using the Kaplan–Meier method and the log-rank test. A P value of <0.05 was considered statistically significant; all tests were two-sided.

Because hepatectomy was not performed on the basis of random assignment in the present study, confounding factors could hamper the observations obtained from unadjusted factors. To reduce the potential bias, a propensity score [24] was calculated to assess the conditional probability of treatment according to the individual's covariates and to balance treatment choice-related variables such that the analysis simulated random assignment [25].

The propensity score was estimated using a logistic regression model in which outcome was the binary variable, severe ALD group versus mild ALD group (0, mild ALD groups; 1, severe ALD group), and the explanatory variables were the independent factors obtained from the multivariate analysis for DFS, such as pathological chronic hepatitis, preoperative serum albumin level, and tumor vascular invasion into the hepatic vein in the analysis of 91 NBNC patients. The propensity score was estimated between the categorizing of severe ALD group versus no-severe ALD group (0, no-severe ALD groups; 1, severe

ALD group) in the analysis of 543 patients. Without replacement, one-to-one pair matching by estimated propensity score generated 15 matched pairs of mild versus severe ALD (Table 4) and 55 matched pairs of patients with and without severe ALD (Supplementary Table 2). All matching processes were performed by the aforementioned SPSS version 21.0. The degree of covariate imbalance in the unmatched and matched samples was measured using the standardized (mean and proportion) difference proposed by Austin et al [26]. It has been suggested that a standardized difference of greater than 20 % represents meaningful imbalance in a given variable between treatment groups [27].

Results

The baseline characteristics of the patients are summarized in Table 1. Liver function of the NBNC patients was, on average, better than that of HCV-infected patients, as judged by the ICG-R15, aspartate aminotransferase level, platelet count, and albumin level ($P < 0.0001$); while the prothrombin time, total bilirubin level, and Child-Pugh scores were comparable. Liver cirrhosis was significantly less frequent in NBNC patients (34 %) than in HCV- (56 %) and HBV- (52 %) infected patients ($P < 0.0001$). The mean tumor size was larger in the NBNC patients than in the HCV-infected patients, whereas the other indices of tumor malignancy, such as microvascular invasion, serum alpha-fetoprotein (AFP) level, and des-gamma-carboxy prothrombin (DCP) level, did not vary significantly. Alcoholism and severe ALD were more evident in NBNC patients than in the other two groups ($P < 0.0001$). The groups did not differ significantly with respect to the pathological surgical margin, although it was noteworthy that non-anatomic resection was frequently selected for HCV-infected patients. As shown in Fig. 1, the DFS rate of NBNC patients was longer than that of HBV and HCV patients. The 5-year DFS rates were 30, 21, and 34 % in the HBV, HCV, and NBNC groups, respectively. The NBNC patients experienced recurrence less frequently than did patients infected with HBV ($P = 0.011$) and HCV ($P < 0.0001$; Fig. 1). We excluded the cases of autoimmune hepatitis and primary biliary cirrhosis from NBNC group.

In univariate analysis of 543 patients, NBNC was an important determinant for good prognosis (HR, 0.6; 95 % CI, 0.4–0.8, $P < 0.0001$), as shown in Supplemental Table 1. The other determinants were liver functional reserve factors (ICG-R15, serum AST, prothrombin time, and serum albumin), tumor factors (size, number, serum tumor marker, vascular invasion), noncancerous liver histology (chronic liver hepatitis and liver cirrhosis), and

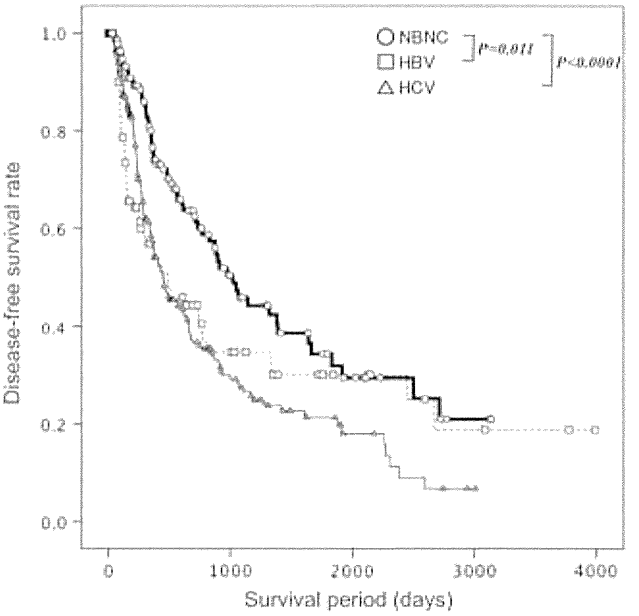


Fig. 1 Disease-free survival of patients with non-B non-C (NBNC) hepatocellular carcinoma. *Open squares*, *triangles*, and *circles* denote the disease-free survival (DFS) of patients with HBV, HCV, and NBNC, respectively. The DFS of the non-B non-C (NBNC) group was better than that of the HBV group ($P = 0.011$) and HCV group ($P < 0.0001$)

surgical factors (anatomic resection, surgical margin). Multivariate analysis revealed that NBNC (HR, 0.5; 95 % CI, 0.4–0.8, $P < 0.0001$), ICG-R15, serum AST, tumor number, vascular invasion, anatomic resection and pathological chronic hepatitis. Taking into account factors related to prognosis, we compared the DFS rate of patients in the presence and absence of the severe ALD, adjusting for the risk factors using propensity score matching. The area under the ROC curves (C value) was 0.892 ± 0.016 SE for predicting severe ALD considering alcoholism. As shown in Supplemental Table 2, all factors related to recurrence were adjusted significantly considering the propensity score constructed with the aforementioned factors. There was no significant difference between the two groups with respect to propensity score after the adjustment ($P = 1.000$), though there was a significant difference before the propensity adjusting ($P < 0.0001$). Younger age, male gender, alcoholism, and higher albumin level observed in the severe ALD group before the matching were completely adjusted after the matching. The DFS rates with and without the severe ALD groups were compared (Supplemental Fig. 1). The 1-, 3-, and 5-year DFS rates were 70, 32, and 32 % in the severe ALD group and 76, 68, and 48 % in the no-severe ALD group, respectively. There was a remarkable difference between the two groups with respect to DFS rates (log-rank; $P = 0.013$). These results suggest that severe

ALD also increases the risk of HCC recurrence amongst all patients with NBNC HCC.

These findings led us to determine which factor decides the DFS rate of NBNC patients. A total of 35 out of 168 NBNC patients were classified as having severe ALD (Table 2). The alcoholic history was available in 163 NBNC patients. Of these 168 patients, 17 patients tested positive for serum HBc antibody (+). Severe ALD was associated with being male ($P = 0.005$), alcoholism ($P < 0.0001$), small tumor size ($P = 0.040$) and liver cirrhosis (f4) ($P = 0.011$). There was no difference in all fibrosis grades except for liver cirrhosis grade (f4) among the three groups. As shown, there was no difference in fibrosis grade (f0–3) among the three groups. There was no significant difference among the groups with respect to any of the other factors. The mean follow-up period after surgery was 2.7 years. As shown in Fig. 2, the 5-year DFS rates were 25.2 and 51.2 % in the severe and mild ALD, respectively ($P = 0.013$). However, the result may be biased by additional determinants of DFS, for example, liver cirrhosis. Liver cirrhosis (f4) was the most evident in the severe ALD group among the three groups, though the liver function was not different and tumor size was the largest in the no-ALD group.

Table 3 summarizes the results of univariate analysis of DFS in 163 NBNC patients (excluding five patients whose alcohol histories were not available), which show that a decreased serum albumin level ($P = 0.033$), tumor number

Table 2 Background characteristics of 168 NBNC patients with alcohol consumption

	Non-B non-C hepatocellular carcinoma			
	Severe ALD ($n = 35$) Mean ± SD	Mild ALD ($n = 56$) Mean ± SD	No ALD ($n = 72$) Mean ± SD	*P*
Age (years)	65.3 ± 8.6	69.2 ± 9.7	69.4 ± 13.2	0.166
Gender				
Male	33/94 %	51/91 %	53/74 %	0.005*
AST (IU/L)	45.8 ± 32.0	40.6 ± 26.9	42.8 ± 24.5	0.677
Platelet (10^4/μL)	17.4 ± 7.5	19.1 ± 9.6	20.7 ± 13.0	0.337
Alcoholism (+)	28/90 %	42/75 %	5/7 %	<0.0001*
HBc antibody (+)	2/6 %	2/4 %	13/22 %	0.017*
Liver function				
ICG-R15 (%)	14.7 ± 8.3	15.9 ± 9.5	15.1 ± 10.3	0.810
Prothrombin time (%)	87.6 ± 12.5	84.7 ± 20.3	86.1 ± 15.4	0.919
Albumin (g/dL)	4.2 ± 0.3	4.0 ± 0.4	4.0 ± 0.5	0.107
Total bilirubin (mg/dL)	0.9 ± 0.4	0.9 ± 0.6	0.9 ± 0.5	0.767
Child-Pugh score	4.4 ± 1.8	4.8 ± 1.6	5.0 ± 1.6	0.175
Tumor factors				
Tumor size (cm)	4.9 ± 3.1	5.2 ± 3.8	6.7 ± 4.5	0.040*
Number	1.5 ± 0.7	1.6 ± 1.7	1.4 ± 1.0	0.611
Alpha-fetoprotein (ng/mL)	2357 ± 8358	435 ± 1758	3923 ± 21024	0.420
DCP (AU/L)	2970 ± 8721	8142 ± 34595	14434 ± 44929	0.296
Anatomic resection (+)	27/77 %	35/63 %	58/81 %	0.062
Pathological findings				
Surgical margin (+)	3/9 %	9/16 %	11/15 %	0.394
No fibrosis (f0) (+)	5/14 %	19/35 %	23/33 %	0.128
Portal fibrosis (f1) (+)	7/20 %	5/9 %	10/14 %	0.354
Bridging fibrosis (f2) (+)	1/3 %	8/15 %	12/17 %	0.110
Distorted lobules (f3) (+)	3/9 %	4/7 %	7/10 %	0.867
Liver cirrhosis (f4) (+)	19/54 %	18/33 %	17/25 %	0.011*
Chronic hepatitis (+)	11/31 %	19/35 %	34/49 %	0.133
Micro-vascular invasion				
vp (+)	11/31 %	23/41 %	34/47 %	0.297
vv (+)	3/9 %	7/14 %	12/19 %	0.410
b (+)	5/14 %	4/7 %	6/8 %	0.489

Values are shown as the mean ± SD. The alcohol history was not available in five patients

b biliary invasion, *DCP* des-gamma-carboxy prothrombin, *pv* portal venous invasion, *vv* hepatic venous invasion

* $P < 0.05$ considered significant

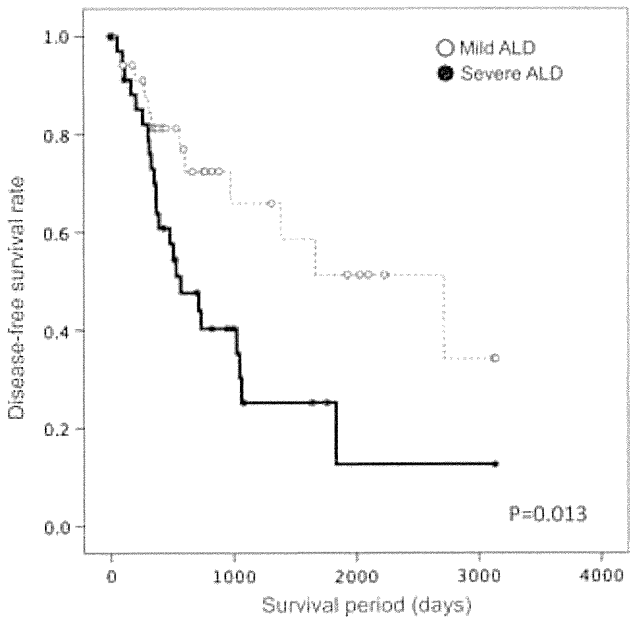


Fig. 2 Disease-free survival of non-B non-C patients in severe and mild alcohol liver disease (ALD) groups before adjustment with propensity scores. *Open* and *closed circles* denote the mild and severe ALD patients, respectively. The difference between the two groups was remarkable ($P = 0.013$)

($P = 0.034$), an elevated serum AFP level ($P = 0.004$), vascular invasion into the hepatic vein ($P = 0.001$), and severe ALD ($P = 0.02$) were possible risk factors. Occult HBV infection was more frequently found in no-ALD patients, although this did not reach statistical significance ($P = 0.223$). Multivariate analysis revealed that vascular invasion into the hepatic vein (HR, 3.3; 95 % CI, 1.7–6.3; $P \leq 0.0001$) and severe ALD (HR, 2.0; 95 % CI, 1.1–3.6; $P = 0.020$) were also risk factors for DFS.

To make a fair comparison, taking into account alcohol consumption as a factor related to prognosis, we adjusted for the risk factors using propensity score matching. As shown in Table 4, all factors related to recurrence were adjusted significantly. There was no significant difference between the two groups with respect to propensity score ($P = 1.000$). For the risk factors examined, we found that standard difference, an index for the imbalance between sample groups, significantly improved from beyond 20 % before adjustment with propensity score matching to within 20 % after adjustment (data not shown). The standardized difference of propensity score before matching (70.9 %) was significantly adjusted after matching (0 %). After adjusting the score, the DFS rates of the severe and mild ALD groups were compared (Fig. 3). The 1-, 3-, and 5-year DFS rates were 84, 64, and 50 % in the mild ALD group and 69, 42, and 26 % in the severe ALD group, respectively. There was a remarkable difference between the two groups with respect to DFS rates (log-rank; $P = 0.035$). These results suggest that severe ALD also increases the risk of HCC recurrence amongst NBNC patients.

Discussion

The present study suggests that preoperative severe ALD increases the risk of HCC recurrence after hepatectomy in HCC patients involving NBNC-derived HCC (Fig. 3, Supplemental Fig. 1) and the DFS rate in patients with NBNC-related HCC was superior to that in patients with HCV- or HBV-related HCC (Fig. 1, Supplemental Table 1). Propensity score matching allowed a fair comparison of the severe ALD and the other groups, as shown in Table 4 and Supplemental Table 2.

In NBNC patients, all of the factors tested for an association with HCC recurrence by multivariate analysis were also adjusted (Table 4). Propensity score in the severe and mild ALD groups were comparable after the matching ($P = 1.000$), though the propensity score value in the severe ALD group was significantly higher than that in the mild ALD group before the matching ($P = 0.002$). Before adjusting for the confounding factors by the matching, we found that being male, alcoholism, relatively small tumor size, and liver cirrhosis were all significantly more common in the severe ALD group (Table 2). As shown in Fig. 2, the 5-year DFS rates in the severe and mild ALD groups were 25 and 51 %, respectively ($P = 0.013$). After adjusting for the prognostic indices (Fig. 3), the difference in the DFS rate between patients who did and did not show severe ALD was not changed (26 vs. 50 %, $P = 0.035$). In NBNC patients, using propensity score matching, we came to this conclusion because the *C*-value to estimate how the score would predict the severe ALD patients was 67 % (95 % CI, 56–78.3 %; $P = 0.007$) (data not shown).

In 543 patients (including HBV, HCV, and NBNC patients), all of the factors tested for an association with HCC recurrence by multivariate analysis were also adjusted (Supplemental Table 2). Propensity scores in the presence and absence of severe ALD were comparable after the matching ($P = 1.000$), though the propensity score in the severe ALD group was significantly higher before the matching ($P < 0.0001$). The C-value of the score estimating the severe ALD patients was 89 % (95 % CI, 86–92 %; $P < 0.0001$). Before adjusting for the confounding factors by the matching, we found that being a younger male, alcoholism, and higher serum albumin were all significantly more common in the severe ALD group (Supplemental Table 2). All factors were adjusted by the propensity score matching. After the adjusting for the prognostic indices (Supplemental Fig. 1), the DFS rates of patients who did and did not show severe ALD were 32 and 48 %, respectively ($P = 0.013$). These results suggest that

Table 3 Univariate and multivariate analysis for the disease-free survival of 168 non-B non-C patients

	Univariate analysis			Multivariate analysis		
	HR	95.0 % CI	*P*	HR	95.0 % CI	*P*
Gender						
Female	0.5	(0.2–1.1)	0.100			
Age (years)						
>71	0.8	(0.5–1.3)	0.327			
Severe ALD (+)	1.8	(1.1–3.1)	0.021*	2.0	(1.1–3.6)	0.020*
Alcoholism (+)	1.1	(0.6–1.7)	0.809			
HBcAb (+)	1.8	(0.7–4.4)	0.223			
Liver functional factor						
ICG-R15 (%)						
>13	1.2	(0.7–2.0)	0.433			
AST (IU/L)						
>34	1.3	(0.8–2.1)	0.317			
Platelet (10^4/μL)						
>17.8	1.4	(0.9–2.3)	0.164			
Prothrombin time (%)						
>86.1	0.8	(0.5–1.4)	0.484			
Albumin (g/dL)						
>4.1	0.6	(0.3–1.0)	0.033*	0.6	(0.4–1.1)	0.109
Total bilirubin						
>0.8	1.2	(0.7–1.9)	0.546			
Child-Pugh score						
>6	1.8	(1.0–3.3)	0.057			
Tumor factor						
Tumor size (cm)						
>5	1.2	(0.7–2.0)	0.543			
Multiple (+)	1.8	(1.0–3.0)	0.034*	1.4	(0.8–2.5)	0.218
AFP (ng/mL)						
>8	2.1	(1.3–3.6)	0.004*	1.6	(0.9–2.7)	0.121
DCP (AU/L)						
>75	1.6	(1.0–2.6)	0.074			
Anatomic resection (+)	0.8	(0.5–1.4)	0.472			
Pathological findings						
Micro-vascular invasion						
vp (+)	1.3	(0.8–2.2)	0.235			
vv (+)	2.9	(1.6–5.4)	0.001*	3.3	(1.7–6.3)	<0.0001*
b (+)	1.7	(0.8–3.8)	0.182			
Surgical margin (+)	1.7	(0.9–3.2)	0.124			
Chronic hepatitis (+)	0.6	(0.3–1.1)	0.088			
Liver cirrhosis (+)	1.3	(0.7–2.3)	0.479			

The alcohol history was not available in five patients

AFP alpha-fetoprotein, *b* biliary invasion, *DCP* des-gamma-carboxy prothrombin, *vp* portal venous invasion, *vv* hepatic venous invasion

* $P < 0.05$ considered significant

severe ALD was a determinant of DFS in those HCC patients.

The present study involved 72 no-ALD patients out of 543 HCC patients (13.3 %), and 21 % of the NBNC patients were diagnosed as having severe ALD (Table 2). The prevalence of NAFLD is reported to be 20 % in Japan with or without HCC [5, 6]. HCCs are found in 10.1 % of patients with alcohol-induced cirrhosis, whereas HCCs were identified in 14–19 % of patients without cirrhosis in Western countries [28–30]. In a Japanese nationwide study with 54,003 HCC patients, 9,307 patients were classified as having NBNC HCC (17.3 %) and 35 % of them were diagnosed with severe alcoholic disease (more than 86 g/day) [31]. The ratios are higher than the present study. Multivariate analysis in the present study revealed that the severe ALD and tumor invasion into the hepatic vein that

Table 4 Baseline characteristics after the adjustment by propensity score matching in severe and mild ALD patients

	Pre-propensity Score Matching ($N = 91$)			Post-propensity Score Matching ($N = 30$)		
	Severe ALD Mean ± SD	Mild ALD Mean ± SD	P	Severe ALD Mean ± SD	Mild ALD Mean ± SD	P
Propensity score	0.45 ± 0.16	0.34 ± 0.15	0.002*	0.43 ± 0.15	0.43 ± 0.15	1.000
Age (years)	65.3 ± 8.6	69.2 ± 9.7	0.053	66.1 ± 9.0	66.0 ± 10.4	0.958
Male (+)	33/94 %	51/91 %	0.703	28/93 %	26/87 %	0.671
Alcoholism (+)	32/91 %	42/75 %	0.058	27/90 %	21/70 %	0.104
ICG-R15 (%)	14.7 ± 8.3	15.9 ± 9.5	0.515	14.6 ± 7.4	13.7 ± 8.7	0.681
AST (IU/L)	45.8 ± 32.0	40.6 ± 26.9	0.412	46.2 ± 33.9	41.1 ± 29.9	0.541
Platelet (10^4/μL)	17.4 ± 7.5	18.3 ± 9.7	0.651	17.3 ± 5.9	19.4 ± 9.6	0.312
PT (%)	87.6 ± 12.5	84.7 ± 20.3	0.464	88.1 ± 13.4	85.9 ± 19.6	0.614
Albumin (g/dL)	4.2 ± 0.3	4.0 ± 0.4	0.014*	4.1 ± 0.3	4.1 ± 0.4	0.649
T-Bil (mg/dL)	0.9 ± 0.4	0.9 ± 0.6	0.574	0.8 ± 0.4	0.9 ± 0.4	0.767
Child Pugh score	4.4 ± 1.8	4.8 ± 1.6	0.231	4.4 ± 1.8	4.6 ± 1.9	0.780
Tumor size (cm)	4.9 ± 3.1	5.2 ± 3.8	0.757	5.0 ± 3.1	5.1 ± 4.4	0.876
Tumor number	1.5 ± 0.7	1.6 ± 1.7	0.690	1.5 ± 0.7	1.5 ± 2.0	0.865
AFP (ng/mL)	2357 ± 8358	435 ± 1758	0.105	2733 ± 9018	589 ± 2341	0.228
DCP (AU/L)	2970 ± 8721	8142 ± 34594	0.396	3420 ± 9388	11084 ± 46756	0.391
Anatomic resection (+)	27/77 %	35/63 %	0.171	23/77 %	21/70 %	0.771
Pathological findings						
vp (+)	11/31 %	23/50 %	0.382	8/27 %	14/47 %	0.180
vv (+)	3/9 %	7/14 %	0.733	3/11 %	2/7 %	1.000
b (+)	5/14 %	4/7 %	0.298	5/17 %	2/7 %	0.424
Surgical margin (+)	9/9 %	9/16 %	0.359	3/10 %	5/17 %	0.706
Chronic hepatitis (+)	11/31 %	19/35 %	0.820	9/30 %	10/36 %	0.781
Liver cirrhosis (+)	19/54 %	18/33 %	0.078	17/57 %	9/32 %	0.071

Values are shown as the mean ± SD

AFP alpha-fetoprotein, *b* biliary invasion, *DCP* des-gamma-carboxy prothrombin, *PT* prothrombin time, *T-Bil* total bilirubin, *vp* portal venous invasion, *vv* hepatic venous invasion

* $P < 0.05$ considered significant

were typicall sufficient in predicting the prognosis of conventional HCC patients with viral infections were also the independent risk factors in NBNC patients. Alcoholism was not found to be a risk factor for the recurrence of HCC in NBNC patients (Table 3). The 5-year DFS rates were 30, 21, and 25 % in the HBV, HCV, and severe ALD groups of the NBNC patients, respectively (Figs. 1, 2), though the 5-year DFS rate was 51 % in the mild ALD group of NBNC patients. The malignant potential of the severe ALD group of NBNC patients may be comparable to that of HBV and HCV patients. Chronic alcohol use in the absence of viral infection significantly increased the risk of HCC by 1.6- to 4-fold when alcohol intake was defined only as drinking, without reference to the amount or frequency of alcohol consumption [32, 33]. The odds ratio increases 5- to 7-fold, especially in patients with an alcohol intake of more than 80 g/day for more than 10 years [34].

The mechanism of carcinogenesis is unknown and may be unique in NBNC patients. In the present study, liver cirrhosis was not found to be an independent determinant for HCC recurrence in multivariate analysis (Table 3), though it was more frequent in the severe ALD group (Table 2). The patients in this study were not exposed to the other chemical agents, such as aflatoxins and exogenous steroids that may cause HCC in NBNC patients. The development of HCC may not always depend on liver inflammation and fibrosis [35, 36]. Occult HBV infection was not associated with the poor prognosis of NBNC patients (Table 3). DFS after hepatectomy in patients with occult HBV infection was comparable with that in patients without occult HBV infection. The 5-year DFS rate in patients with occult HBV infection was 42 % (data not shown). Whether occult HBV infection is involved in NBNC-derived HCC is still controversial [37, 38]. The present study is consistent with the previous report. Liver functional factors did not determine the DFS rate in NBNC patients (Table 3), though the ICG-R15 and serum AST level determined the DFS rate of 543 patients

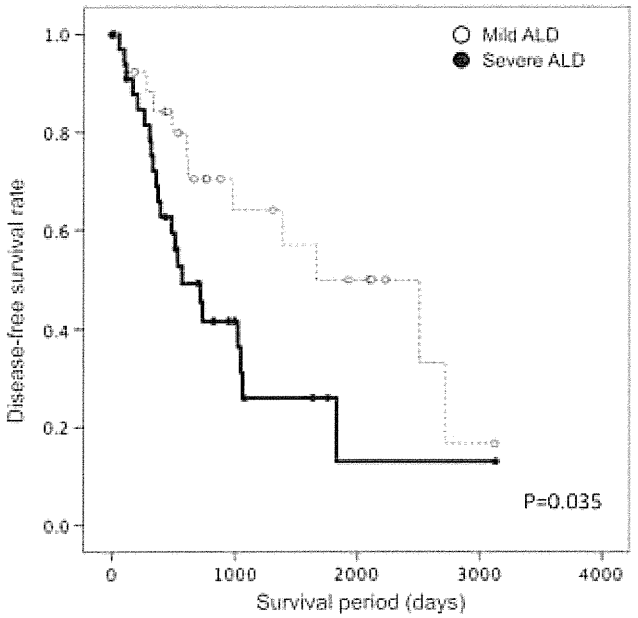


Fig. 3 Disease-free survival rates of non-B non-C patients in severe and mild alcohol liver disease (ALD) groups after adjustment with propensity scores. *Open* and *closed circles* denote the mild and severe ALD groups, respectively. The difference between the two groups was remarkable ($P = 0.035$)

(Supplemental Table 1). The liver function of NBNC patients was significantly better than that of HCV patients (Table 1). Good liver function at the initial hepatectomy may prevent early recurrence in patients with NBNC HCC without abusive alcohol consumption [2]. Such patients may have better liver function without the chronic active inflammation seen in HBV- or HCV-infected patients [39, 40].

Limitations of the present study include that the data of genome-wide gene expression and the data of urinary constituents were not available to elucidate the mechanisms of carcinogenesis in NBNC livers in the presence or absence of severe ALD. Multicentric occurrence of HCC is also associated with reduced levels of sirtuin 3, a protein that regulates hepatocellular orotic acid concentration and inhibits hepatic carcinogenesis [41, 42]. Genome-wide gene expression analysis of liver samples indicated that the multicentric occurrence of HCC was associated with decreased *SLC22A7* expression, leading to a reduction in the transportation of orotic acid [41]. Adult male alcoholics are found to have elevated urinary orotic acid levels that decline with time following abstinence [43]. An experimental study provided evidence that alcoholism and various other diseases alter hepatocellular excretion of orotic acids, which can promote liver carcinogenesis after partial hepatectomy [44]. Further research is needed to fully elucidate the mechanisms that underlie liver carcinogenesis.

In conclusion, HCC was found to recur less frequently in the cases of NBNC HCC than in the cases of HCC with viral infection. Moreover, preoperative severe ALD was strongly associated with HCC recurrence after hepatectomy in NBNC patients.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Suzuki Y, Ohtake T, Nishiguchi S, Hashimoto E, Aoyagi Y, Onji M, et al. Survey of non-B, non-C liver cirrhosis in Japan. Hepatol Res Off J Japan Soc Hepatol. 2012. (Epub 2013/01/26).
2. Yokoi Y, Suzuki S, Baba S, Inaba K, Konno H, Nakamura S. Clinicopathological features of hepatocellular carcinomas (HCCs) arising in patients without chronic viral infection or alcohol abuse: a retrospective study of patients undergoing hepatic resection. J Gastroenterol. 2005;40(3):274–82 (Epub 2005/04/15).
3. Takenaka K, Yamamoto K, Taketomi A, Itasaka H, Adachi E, Shirabe K, et al. A comparison of the surgical results in patients with hepatitis B versus hepatitis C-related hepatocellular carcinoma. Hepatology (Baltimore, Md). 1995;22(1):20–4 (Epub 1995/07/01).
4. Uetake S, Yamauchi M, Itoh S, Kawashima O, Takeda K, Ohata M. Analysis of risk factors for hepatocellular carcinoma in patients with HBs antigen- and anti-HCV antibody-negative alcoholic cirrhosis: clinical significance of prior hepatitis B virus infection. Alcohol Clin Exp Res. 2003;27(8 Suppl):47S–51S (Epub 2003/09/10).
5. Saibara T. Nonalcoholic steatohepatitis in Asia-Oceania. Hepatol Res Off J Jpn Soc Hepatol. 2005;33(2):64–7 (Epub 2005/11/01).
6. Yoshiike N, Lwin H. Epidemiological aspects of obesity and NASH/NAFLD in Japan. Hepatol Res Off J Jpn Soc Hepatol. 2005;33(2):77–82 (Epub 2005/10/18).
7. Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology. 2005;129(1):113–21 (Epub 2005/07/14).
8. Ekstedt M, LE Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology (Baltimore, Md). 2006;44(4):865–73 (Epub 2006/09/29).
9. Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. J Hepatol. 2008;49(4):608–12 (Epub 2008/08/07).
10. Rafiq N, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, et al. Long-term follow-up of patients with nonalcoholic fatty liver. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2009;7(2):234–8 (Epub 2008/12/04).
11. Rabe C, Pilz T, Klostermann C, Berna M, Schild HH, Sauerbruch T, et al. Clinical characteristics and outcome of a cohort of 101 patients with hepatocellular carcinoma. World J Gastroenterol WJG. 2001;7(2):208–15 (Epub 2002/01/31).
12. Kaczynski J, Hansson G, Hermodsson S, Olsson R, Wallerstedt S. Minor role of hepatitis B and C virus infection in the etiology of hepatocellular carcinoma in a low-endemic area. Scand J Gastroenterol. 1996;31(8):809–13 (Epub 1996/08/01).
13. Hellerbrand C, Hartmann A, Richter G, Knoll A, Wiest R, Scholmerich J, et al. Hepatocellular carcinoma in southern

Germany: epidemiological and clinicopathological characteristics and risk factors. Dig Dis (Basel, Switzerland). 2001;19(4):345–51 (Epub 2002/04/06).
14. De Bac C, Stroffolini T, Gaeta GB, Taliani G, Giusti G. Pathogenic factors in cirrhosis with and without hepatocellular carcinoma: a multicenter Italian study. Hepatology (Baltimore, Md). 1994;20(5):1225–30 (Epub 1994/11/01).
15. Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol. 2002;155(4):323–31 (Epub 2002/02/12).
16. Shiraishi M, Hiroyasu S, Nagahama M, Tomita S, Miyahira T, Kusano T, et al. Characteristics of hepatocellular carcinoma in patients with negative virus markers: clinicopathologic study of resected tumors. World J Surg. 1999;23(3):301–5 (Epub 1999/02/06).
17. Noguchi K, Nakashima O, Nakashima Y, Shiota K, Nawata H, Kojiro M. Clinicopathologic study on hepatocellular carcinoma negative for hepatitis B surface antigen and antibody to hepatitis C virus. Int J Mol Med. 2000;6(6):661–5 (Epub 2000/11/18).
18. Kaibori M, Ishizaki M, Matsui K, Kwon AH. Clinicopathologic characteristics of patients with non-B non-C hepatitis virus hepatocellular carcinoma after hepatectomy. Am J Surg. 2012;204(3):300–7 (Epub 2012/05/18).
19. Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Brechot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? Hepatology (Baltimore, Md). 2001;34(1):194–203 (Epub 2001/06/30).
20. Torbenson M, Thomas DL. Occult hepatitis B. Lancet Infect Dis. 2002;2(8):479–86 (Epub 2002/08/02).
21. Sato K, Tanaka S, Mitsunori Y, Mogushi K, Yasen M, Aihara A, et al. Contrast-enhanced intraoperative ultrasonography for vascular imaging of hepatocellular carcinoma; clinical and biological significance. Hepatology (Baltimore, Md). 2012. (Epub 2012/11/15).
22. Mitsunori Y, Tanaka S, Nakamura N, Ban D, Irie T, Noguchi N, et al. Contrast-enhanced intraoperative ultrasound for hepatocellular carcinoma: high sensitivity of diagnosis and therapeutic impact. J Hepato Biliary Pancreat Sci. 2012. (Epub 2012/03/09).
23. Morse RM, Flavin DK. The definition of alcoholism. The Joint Committee of the National Council on Alcoholism and Drug Dependence and the American Society of Addiction Medicine to Study the Definition and Criteria for the Diagnosis of Alcoholism. JAMA J Am Med Assoc. 1992;268(8):1012–4 (Epub 1992/08/26).
24. Little RJ, Rubin DB. Causal effects in clinical and epidemiological studies via potential outcomes: concepts and analytical approaches. Annu Rev Public Health. 2000;21:121–45 (Epub 2000/07/08).
25. Ruzzenente A, Guglielmi A, Sandri M, Campagnaro T, Valdegamberi A, Conci S, et al. Surgical resection versus local ablation for HCC on cirrhosis: results from a propensity case-matched study. J Gastrointest Surg Off J Soc Surg Alimentary Tract. 2012;16(2):301–11 (discussion 11. Epub 2011/11/19).
26. Austin PC, Grootendorst P, Anderson GM. A comparison of the ability of different propensity score models to balance measured variables between treated and untreated subjects: a Monte Carlo study. Stat Med. 2007;26(4):734–53 (Epub 2006/05/19).
27. Cochran WG, Rublin DB. Controlling bias in observational studies: a review. Sankhya Series A. 1973;35:417–46.
28. Nzeako UC, Goodman ZD, Ishak KG. Hepatocellular carcinoma in cirrhotic and noncirrhotic livers. A clinico-histopathologic study of 804 North American patients. Am J Clin Pathol. 1996;105(1):65–75 (Epub 1996/01/01).
29. Chiesa R, Donato F, Tagger A, Favret M, Ribero ML, Nardi G, et al. Etiology of hepatocellular carcinoma in Italian patients with and without cirrhosis. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2000;9(2):213–6 (Epub 2000/03/04).
30. Grando-Lemaire V, Guettier C, Chevret S, Beaugrand M, Trinchet JC. Hepatocellular carcinoma without cirrhosis in the West: epidemiological factors and histopathology of the non-tumorous liver. Groupe d'Etude et de Traitement du Carcinome Hepatocellulaire. J Hepatol. 1999;31(3):508–13. (Epub 1999/09/17).
31. Utsunomiya T, Shimada M, Kudo M, Ichida T, Matsui O, Izumi N, et al. Nationwide study of 4741 patients with non-B non-C hepatocellular carcinoma with special reference to the therapeutic impact. Ann Surg. 2013. (Epub 2013/05/16).
32. Chen CJ, Liang KY, Chang AS, Chang YC, Lu SN, Liaw YF, et al. Effects of hepatitis B virus, alcohol drinking, cigarette smoking and familial tendency on hepatocellular carcinoma. Hepatology (Baltimore, Md). 1991;13(3):398–406 (Epub 1991/03/01).
33. Yu MW, You SL, Chang AS, Lu SN, Liaw YF, Chen CJ. Association between hepatitis C virus antibodies and hepatocellular carcinoma in Taiwan. Cancer Res. 1991;51(20):5621–5 (Epub 1991/10/15).
34. Morgan TR, Mandayam S, Jamal MM. Alcohol and hepatocellular carcinoma. Gastroenterology. 2004;127(5 Suppl 1):S87–96 (Epub 2004/10/28).
35. Alpert ME, Hutt MS, Wogan GN, Davidson CS. Association between aflatoxin content of food and hepatoma frequency in Uganda. Cancer. 1971;28(1):253–60 (Epub 1971/07/01).
36. Kew MC, Popper H. Relationship between hepatocellular carcinoma and cirrhosis. Semin Liver Dis. 1984;4(2):136–46 (Epub 1984/05/01).
37. Tamori A, Nishiguchi S, Kubo S, Narimatsu T, Habu D, Takeda T, et al. HBV DNA integration and HBV-transcript expression in non-B, non-C hepatocellular carcinoma in Japan. J Med Virol. 2003;71(4):492–8 (Epub 2003/10/14).
38. Kannangai R, Molmenti E, Arrazola L, Klein A, Choti M, Thomas DL, et al. Occult hepatitis B viral DNA in liver carcinomas from a region with a low prevalence of chronic hepatitis B infection. J Viral Hepatitis. 2004;11(4):297–301 (Epub 2004/07/03).
39. Miyagawa S, Kawasaki S, Makuuchi M. Comparison of the characteristics of hepatocellular carcinoma between hepatitis B and C viral infection: tumor multicentricity in cirrhotic liver with hepatitis C. Hepatology (Baltimore, Md). 1996;24(2):307–10 (Epub 1996/08/01).
40. Wu CC, Ho WL, Chen JT, Tang JS, Yeh DC, P'Eng FK. Hepatitis viral status in patients undergoing liver resection for hepatocellular carcinoma. Br J Surg. 1999;86(11):1391–6 (Epub 1999/12/03).
41. Kudo A, Mogushi K, Takayama T, Matsumura S, Ban D, Irie T, et al. Mitochondrial metabolism in the noncancerous liver determine the occurrence of hepatocellular carcinoma: a prospective study. J Gastroenterol. 2013. (Epub 2013/04/02).
42. Zhang YY, Zhou LM. Sirt3 inhibits hepatocellular carcinoma cell growth through reducing Mdm2-mediated p53 degradation. Biochem Biophys Res Commun. 2012;423(1):26–31 (Epub 2012/05/23).
43. Visek WJ, Shoemaker JD. Orotic acid, arginine, and hepatotoxicity. J Am Coll Nutr. 1986;5(2):153–66 (Epub 1986/01/01).
44. Laconi E, Vasudevan S, Rao PM, Rajalakshmi S, Pani P, Sarma DS. The development of hepatocellular carcinoma in initiated rat liver after a brief exposure to orotic acid coupled with partial hepatectomy. Carcinogenesis. 1993;14(12):2527–30 (Epub 1993/12/01).

HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

Visualization of Stem Cell Features in Human Hepatocellular Carcinoma Reveals *In Vivo* Significance of Tumor-Host Interaction and Clinical Course

Shunsuke Muramatsu,[1] Shinji Tanaka,[1] Kaoru Mogushi,[2] Rama Adikrisna,[1] Arihiro Aihara,[1] Daisuke Ban,[1] Takanori Ochiai,[1] Takumi Irie,[1] Atsushi Kudo,[1] Noriaki Nakamura,[1] Koh Nakayama,[3] Hiroshi Tanaka,[2] Shoji Yamaoka,[4] and Shigeki Arii[1]

Hepatocellular carcinoma (HCC) is one of the most aggressive malignancies because of recurrence and/or metastasis even after curative resection. Emerging evidence suggests that tumor metastasis and recurrence might be driven by a small subpopulation of stemness cells, so-called cancer stem cells (CSCs). Previous investigations have revealed that glioma and breast CSCs exhibit intrinsically low proteasome activity and that breast CSCs also reportedly contain a lower reactive oxygen species (ROS) level than corresponding nontumorigenic cells. Here we visualized two stem cell features, low proteasome activity and low intracellular ROS, in HCC cells using two-color fluorescence activated cell sorting to isolate cells with stem cell features. These cells were then analyzed for their division behavior in normoxia and hypoxia, expression of stem cell markers, tumorigenicity, metastatic potential, specific gene expression signatures, and their clinical implications. A visualized small subpopulation of HCC cells demonstrated asymmetric divisions. Their remarkable tumorigenicity in nonobese diabetic/severe combined immunodeficient mice suggested the cancer initiation potential of these HCC CSCs. Comprehensive gene expression analysis revealed that chemokine-related genes were up-regulated in the CSCs subpopulation. Our identified HCC CSCs facilitated the migration of macrophages *in vitro* and demonstrated metastatic potential by way of recruitment of macrophages *in vivo*. In patients who undergo curative operation for HCC, the CSC-specific gene signature in the liver microenvironment significantly correlates with recurrence. *Conclusion*: Based on these findings, the stem cell feature monitoring system proposed here is a promising tool to analyze the *in vivo* significance of CSC microenvironments in human HCCs. (HEPATOLOGY 2013;58:218-228)

Hepatocellular carcinoma (HCC) is one of the most common malignancies and the third leading cause of cancer death worldwide.[1] The primary curative treatment for HCC is surgical resection; however, even after curative resection patient prognosis remains poor because of frequent recurrence and/or metastasis.[2,3] Because cancer stem cells (CSCs) possess self-renewal capacity, multilineage potency, and increased tumorigenicity, it has been hypothesized that CSCs exist as a small population within the bulk tumors and play a critical role in cancer progression, metastasis, and recurrence.[4] Various tools have been reported for identification of the CSC population, including the cell surface markers CD44, CD133, CD90, and ESA/EpCAM.[5-8] In addition, specific stemness properties based on stem cell biology of their

Abbreviations: CSC, cancer stem cells; FDR, false discovery rate; GSEA, gene set enrichment analysis; HCC, hepatocellular carcinoma; NOD/SCID, nonobese diabetic / severe combined immunodeficient; ODC, ornithine decarboxylase; ROS, reactive oxygen species.

From the [1]Department of Hepato-Biliary-Pancreatic Surgery, Graduate School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan; [2]Department of Computational Biology, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan; [3]Oxygen Biology Unit, Frontier Research Laboratory, Medical Research Institute Tokyo Medical and Dental University, Tokyo, Japan; [4]Department of Molecular Virology, Tokyo Medical and Dental University, Tokyo, Japan.

Received June 30, 2012; accepted February 13, 2013.

Supported by Grant-in-Aid for Scientific Research on Innovative Areas, Scientific Research (A), Project of Development of Innovative Research on Cancer Therapeutics from Ministry of Education, Culture, Sports, Science & Technology of Japan, and Health & Labour Sciences Research Grant from Ministry of Health Labour & Welfare of Japan.