than mere histological staging of F1, F2, F3 or F4. The reproducibility was confirmed by the remaining 276 patients' data obtained from the other seven hospitals. Although the validation data were collected from different geographic area and different chronologic situation, FSC showed similar results in prediction of histological staging.

Fibrotic score for hepatitis C seemed a very useful quantitative marker in evaluating severity of fibrotic severity of hepatitis C patients without invasive procedures and without any specialized ultrasonography or magnetic resonance imaging. FSC also has an advantage of measurement, in which old blood samples are available for retrospective assessment of varied clinical settings: old sera from 20 years ago at the time of initial liver biopsy, or paired sera before and after a long-term anti-inflammatory therapy, for example. These kinds of retrospective assessments of fibrotic staging will be valuable in estimating a long-term progression of liver disease, in evaluating efficacy of a long-term medication or other medical intervention, or in making a political judgment from the viewpoint of socioeconomic efficacy.

The score can be calculated for any patients with chronic HCV infection. Although this multiple regression model dealt with appropriate logarithmic transformation for non-normal distribution parameters, the regression analysis was based on a linear regression model. Very slight fibrosis can be calculated as less than 1.00, which is commonly found with a slight degree of chronic hepatitis with a tiny fibrotic change as F0. Very severe fibrosis may be calculated as more than 4.00, which is an imaginable and nonsense number in the scoring system of fibrosis. FSC is, however, very useful and valuable in real clinical setting. Estimation of severity of liver fibrosis in outpatient clinics, evaluation of natural progression of patients' fibrosis over 10 years, and assessment of a long-term administration of interferon in patients with chronic hepatitis C from the viewpoint of fibrotic change. In this study, because certain patients actually had a history of interferon administration, regression of liver fibrosis during and after the treatment could be assessed when prior sera were available for serial evaluation of FSC. We can also expect the usefulness of evaluation of carcinogenic risk after sustained virological response, and stage progression with alcohol intake or obesity-induced steatosis. Recent development of new directly acting antiviral agents require evaluation for long-term histological advantage, for aggravation of hepatitis stage during viral and biochemical breakthrough caused by HCV mutation, estimation of future carcinogenic risk, and even for the best way of management of patients with chronic hepatitis C. FSC seems one of the ideal methods of approximation for fibrotic stage of chronic hepatitis C. Repeated measurement is quite suitable for patients with an unestablished treatment or trial, every 1 or 2 years, for example. Because the current regression function was generated from the data of HCV-related chronic liver disease, this equation would not be suitable for the recognition of HBV-related chronic liver disease,[22] alcoholic liver disease and other congenital or autoimmune liver diseases. To recognize the latter diseases, other studies about individual diseases must be performed.

We compared the usefulness of the FSC with that of other fibrotic scores.[8,9,12,13] More simple and inexpensive AAR or APRI could not well estimate fibrotic stages with poor correlation coefficients of 0.021 and 0.462, which were much lower than the coefficient of FSC of 0.572. FibroTest, which contained three costly fibrotic markers (α2-macroglobulin, haptoglobin and apolipoprotein A1), also showed a low correlation coefficient of 0.415, suggesting that the usefulness was limited in HCV positive Asian patients. Although FIB-4 demonstrated the best coefficient of 0.440 among the fibrotic scores, significant overlaps were found between neighboring stages and obtained scores were not coordinated for rea17l histological classification. Because this study also measured those special markers included in FibroTest, the ability of discrimination of fibrotic stages could be compared among the five fibrotic scoring systems.

In conclusion, FSC was a useful and reliable biomarker for prediction of liver fibrosis in patients with chronic HCV infection. FSC is expected to be introduced and utilized in varied kinds of studies and trials. Its accuracy and reproducibility require further validation using more numbers of patients in several countries other than Japan.

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REFERENCES

1 Sandrin L, Fourquet B, Hasquenoph JM *et al.* Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; 29: 1705–13.
2 Hanna RF, Kased N, Kwan SW *et al.* Double-contrast MRI for accurate staging of hepatocellular carcinoma in patients with cirrhosis. *AJR Am J Roentgenol* 2008; 190: 47–57.

3 Hagiwara M, Rusinek H, Lee VS *et al.* Advanced liver fibrosis: diagnosis with 3D whole-liver perfusion MR imaging – initial experience. *Radiology* 2008; 246: 926–34.
4 Taouli B, Chouli M, Martin AJ, Qayyum A, Coakley FV, Vilgrain V. Chronic hepatitis: role of diffusion-weighted imaging and diffusion tensor imaging for the diagnosis of liver fibrosis and inflammation. *J Magn Reson Imaging* 2008; 28: 89–95.
5 Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. *Gastroenterology* 1988; 95: 734–9.
6 Poynard T, Bedossa P. Age and platelet count: a simple index for predicting the presence of histological lesions in patients with antibodies to hepatitis C virus. METAVIR and CLINIVIR Cooperative Study Groups. *J Viral Hepat* 1997; 4: 199–208.
7 Sheth SG, Flamm SL, Gordon FD, Chopra S. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 1998; 93: 44–8.
8 Giannini E, Botta F, Fasoli A *et al.* Progressive liver functional impairment is associated with an increase in AST/ALT ratio. *Dig Dis Sci* 1999; 44: 1249–53.
9 Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T, MULTIVIRC Group. Biochemical imarkers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; 357: 1069–75.
10 Wai CT, Greenson JK, Fontana RJ *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; 38: 518–26.
11 Benlloch S, Berenguer M, Prieto M, Rayón JM, Aguilera V, Berenguer J. Prediction of fibrosis in HCV-infected liver transplant recipients with a simple noninvasive index. *Liver Transpl* 2005; 11: 456–62.
12 Chrysanthos NV, Papatheodoridis GV, Savvas S *et al.* Aspartate aminotransferase to platelet ratio index for fibrosis evaluation in chronic viral hepatitis. *Eur J Gastroenterol Hepatol* 2006; 18: 389–96.
13 Sterling RK, Lissen E, Clumeck N *et al.* Develoment of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; 43: 1317–25.
14 Alsatie M, Kwo PY, Gingerich JR *et al.* A multivariable model of clinical variables predicts advanced fibrosis in chronic hepatitis C. *J Clin Gastroenterol* 2007; 41: 416–21.
15 Attallah AM, Abdallah SO, El Sayed AS *et al.* Non-invasive predictive score of fibrosis stages in chronic hepatitis C patients based on epithelial membrane antigen in the blood in combination with routine laboratory markers. *Hepatol Res* 2011; 41: 1075–84.
16 Kalantari H, Hoseini H, Babak A, Yaran M. Validation of hepascore as a predictor of liver fibrosis in patients with chronic hepatitis C infection. *Hepat Res Treat* 2011; 2011: 972759.
17 Engstrom-Laurent A, Loof L, Nyberg A, Schroder T. Increased serum levels of hyaluronate in liver disease. *Hepatology* 1985; 5: 638–42.
18 Murawaki Y, Ikuta Y, Koda M, Kawasaki H. Serum type III procollagen peptide, type IV collagen 7S domain, central triple-helix of type IV collagen and tissue inhibitor of metalloproteinases in patients with chronic viral liver disease: relationship to liver histology. *Hepatology* 1994; 20: 780–7.
19 Fabris C, Falleti E, Federico E, Toniutto P, Pirisi M. A comparison of four serum markers of fibrosis in the diagnosis of cirrhosis. *Ann Clin Biochem* 1997; 34: 151–5.
20 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading, and staging. *Hepatology* 1994; 19: 1513–20.
21 IBM SPSS Inc. IBM SPSS for Windows. Version 19.0 manual. SPSS Japan Inc., an IBM company. Armonk NY, USA, 2009.
22 Ikeda K, Izumi N, Tanaka E *et al.* Fibrosis score consisting of four serum markers successfully predicts pathological fibrotic stages of chronic hepatitis B. *Hepatol Res* 2012; 43: 596–604.

Review

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Recent Progress in Radiofrequency Ablation Therapy for Hepatocellular Carcinoma

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Key Words
Hepatocellular carcinoma · Radiofrequency ablation · Bipolar radiofrequency ablation · Dexmedetomidine · Sonazoid · Fusion imaging · Microwave · Surgery

Abstract
In order to attain better ablation and more effective management of hepatocellular carcinoma (HCC), new approaches and devices in radiofrequency ablation (RFA) therapy were presented and discussed in a workshop at the 50th Annual Meeting of the Liver Cancer Study Group of Japan. A novel bipolar RFA apparatus was introduced in Japan in January 2013. Hundreds of subjects with HCC were treated with multipolar RFA with varied devices and plans. Among these, no-touch ablation was one of the most useful procedures in the treatment of HCC with the apparatus. In RFA therapy, a few assisting devices and techniques were applied for convenience and improvement of the thermal ablation procedure. Contrast-enhanced ultrasonography and three-dimensional fusion imaging technique using volume data of CT or MRI could improve exact targeting and shorten the treatment time for RFA procedures under ultrasonographic guidance. A more complicated method using a workstation was also reported as being helpful in planning the ablated shape and volume in multineedle RFA. The effective use of sedatives and antianalgesics as well as a novel microwave apparatus with a cooled-tip electrode was also discussed.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide, with an annual incidence of one million new cases [1]. In about 90% of the patients, HCC is a late complication of cirrhosis. The 5-year incidence of HCC in patients with cirrhosis is 15–20%. The risk of developing HCC has been reported to be 0.5% per year for hepatitis B and 5% per year for hepatitis C patients. Consequently, HCC is now emerging as a major health concern for the next decades. Most patients develop few symptoms when the tumor is small and often present with multifocal disease only at a late stage. The natural course of HCC includes progressive tumor growth compromising the hepatic function, intrahepatic metastases and spread to distant sites. In general, HCC has a poor prognosis, with a median survival of 3–6 months after the onset of symptoms. Nowadays, an increasing number of HCCs is discovered at an early stage because of an increasing awareness and screening of asymptomatic patients with cirrhosis [2]. During the last decade, percutaneous locoregional therapy has become the predominant treatment for a small HCC associated with cirrhosis because of poor liver function reserve and a high recurrence rate after surgical resection.

Radiofrequency ablation (RFA) therapy [3] became available in 1999 in Japan, and turned into an indispensable procedure for the management of small-sized HCC. RFA therapy is especially useful for patients with recurrent tumors, bilobar tumors and tumors located deep in the liver. It is also effective in elderly patients and in patients with other concomitant diseases and cardiac, renal, lung or neurological complications. In the last 15 years, many devices and studies have been described in order to better manage the early stages of HCC.

At the 50th Annual Meeting of the Liver Cancer Study Group of Japan (congress president: Prof. Masatoshi Kudo), a workshop was conducted regarding the recent progress in RFA, which was presented and discussed by 11 hepatology experts (for a summary, see below).

Current Status of RFA

A complete tumor ablation rate of over 90% was achieved with RFA, and the remainder can usually be ablated with additional RFA therapy. Although the tumor recurrence rate or the residual tumor rate is significantly higher after RFA compared with surgical resection, overall survival is generally regarded as not significantly different between RFA and surgery.

Sunakozaka and colleagues compared the prognosis after surgical resection [4, 5] (n = 157) with that after RFA (n = 363) using propensity scores with the inverse probability of treatment weighting method. After selection bias regarding the treatment type (RFA or resection) was adjusted for to some extent, the survival rates between patients with RFA and resection were not different retrospectively. The odds ratio for survival of the propensity score was 1.15 (favoring surgical resection, 95% CI 0.56–2.28). These experts found that the survival period seemed prolonged when patients with decreased liver function received RFA therapy instead of surgery. A multicenter prospective randomized controlled trial (the SURF study) will show the real efficiency of RFA in recurrence-free survival and overall survival in the Japanese clinical setting.

Some surgeons have reported that recurrent or residual tumor tissue after RFA might have malignant characteristics in the pathology of resected HCCs. Saito and colleagues analyzed the clinicopathological features of 10 HCC patients who showed local recurrence after RFA therapy. They selected retrospective patient data as well as searched at the surgical department for biased data and compared them with data of 78 HCC patients without previous RFA intervention. The incidence of poorly differentiated histology (4/10 vs. 72/78, $p < 0.01$) and portal vein invasion (8/10 vs. 13/78, $p < 0.01$) was significantly higher in the HCC patients with previous RFA therapy than in the treatment-naïve HCC patients. The survival rate was significantly lower ($p = 0.03$) and the disease-free survival rate lower ($p = 0.01$) in the patients undergoing RFA therapy. The investigators also noticed a high incidence of extrahepatic metastases (3/10 vs. 6/78, $p < 0.05$) after surgical resection of recurrent HCC after RFA therapy. They analyzed angiogenesis markers (HIF-1, VEGF), cancer stem cell markers (EpCAM, CD44) [6, 7] and epithelial-mesenchymal transition (EMT) markers (TGF-β, twist, snail-1, vimentin) in RT-PCR of the patient serum and in immunohistochemistry of the resected specimen. Since almost all angiogenesis markers, stem cell markers and EMT markers increased to some extent in HCC with previous RFA therapy, the investigators supposed that insufficient thermal ablation led to a malignant transformation with EMT. Since treatment-naïve and treatment-resistant HCCs after the other modalities of therapy sometimes also show these malignant characteristics to some

extent, the analysis of the limited number of selected patients in the surgical department should be carefully interpreted considering the presence of significant data bias.

Monopolar and Bipolar Ablation

In Japan, monopolar RFA treatment (cooled-tip, LeVeen or RITA system) became available in 1999 and bipolar RFA treatment (CelonPOWER) in 2013. More than 50 institutions and hospitals currently use the bipolar system.

Kawamura and colleagues presented various advantages of the bipolar RFA system, especially the no-touch ablation procedure. Treatment benefit is mostly obtained in subcapsular tumors [8], tumor nodules just behind major vascular structure, potentially poorly differentiated tumors, and irregularly shaped nodules. Since other types of HCC nodules also showed a decreased dissemination risk with the treatment, 44 of 130 nodules (34%) were treated with no-touch ablation.

Joko and colleagues reported on 174 patients undergoing bipolar RFA. More than 70% of the treatments were performed using 3 electrodes, and more than half of the nodules were treated with no-touch ablation. These experts revealed that current bipolar apparatus could treat tumors as large as 3.5 cm in diameter with the no-touch system using 3 needles (6 electrodes) around the nodules. They proposed to extend the indication for RFA to cases where multipolar ablation is performed appropriately, except for small-sized HCC.

On the contrary, Nasu and colleagues studied the ideal use of single-needle ablation with the bipolar RFA system. They tried to obtain a small and round ablative area for a small HCC nodule using a single 20-mm applicator (T20) and compared two types of radiofrequency output programs: a conventional protocol with constant 20-Watt output to 10 kJ energy (n = 9) and a step-up protocol with increasing power from 10 W to 3 kJ, from 15 W to 6 kJ and from 20 W to 10 kJ (n = 6). The longitudinal length of the ablated area was the same in the two protocols; however, the transverse length was significantly longer in the step-up protocol ($p = 0.0015$). Although the step-up protocol required a longer treatment time, the revised manner of output control generated a more round and larger necrotic area. Nasu and colleagues therefore recommended the step-up control of output for a small-sized HCC using a 20-mm applicator.

Nakanishi and colleagues reported the usefulness of bipolar RFA in a patient with a cardiac pacemaker comparing monopolar and bipolar ablation. The patient showed a significant decrease in blood pressure with monopolar ablation but did not show any blood pressure change with the bipolar RFA procedure.

Application and Assisting Devices

When RFA is performed under ultrasonographic guidance, there are several reasons for the difficulty in targeting and inserting a needle to an exact point of an HCC nodule, e.g. a small lesion of less than 1 cm, invisible or vague nodules, concomitant confusing or misleading nodular lesions around the tumor and local tumor progression of a previously ablated lesion. When a small nodular lesion is detected on CT or MRI during the follow-up period after ablation therapy or surgical resection, ultrasonography sometimes cannot demarcate the lesion.

Uchino and colleagues studied patients with HCC tumors that are hard to visualize on B-mode ultrasonography. Sonazoid-enhanced ultrasonography was performed in 107 patients with 140 lesions that were vague and invisible on ordinary ultrasound. A total of 140 sessions of RFA therapy were performed under contrast-enhanced ultrasound, and an electrode was inserted in the Kupffer phase in 109 sessions (77.9%), in the arterial phase in 14 sessions (10.0%), and in the ordinary B-mode in the remaining 17 sessions (12.1%). In 114 cases (88.4%), an effective enhancement was achieved at the first session of RFA, and in these complete ablation was attained in 97.4%. Sonazoid-enhanced ultrasonography was useful in small-sized lesions, subcapsular lesions, lesions neighboring previous ablated areas and lesions requiring an additional ablation. On the contrary, sonazoid enhancement was often ineffective in obese patients and patients with a shrunk liver.

Minami and colleagues retrospectively analyzed difficult-to-visualize tumors on B-mode ultrasonography. They compared fusion imaging assistance, enhanced ultrasonography assistance and both fusion imaging plus enhanced ultrasonography assistance in the treatment of RFA. Enhanced ultrasonography was additionally performed when fusion imaging was ineffective in demarcating a tumor, and vice versa. Since the number of required treatment sessions, local tumor progression rate and incidence of adverse events were not different among the three treatment groups, fusion imaging and contrast-

enhanced ultrasonography are regarded as mutually complementary and useful assisting devices in RFA therapy. In addition, Nakanishi and colleagues stated that fusion imaging and sonazoid-enhanced ultrasonography in RFA are helpful and improve the certainty of the ablation procedure.

Sano and colleagues proposed various devices to improve RFA therapy with the bipolar system. The ablation area and shape after treatment were evaluated three-dimensionally using workstation software, and simulation techniques seemed helpful in an appropriate bipolar ablation procedure even after treatment. The ultrasonography function of the Virtu-TRAX™ (GE Healthcare) can indicate the exact depth of multiple inserted needles and helps to perform a sufficient and safe ablation.

Analgesic Agents

In the treatment of RFA, most Japanese doctors use morphine, pethidine, pentazocine or fentanyl as antianalgesics and midazolam or diazepam as sedatives instead of general anesthesia. The administration of these conventional analgesics is sometimes associated with insufficient analgesic effects, and sedatives may cause serious or life-threatening breathing problems such as shallow, slowed or temporarily stopped breathing. Since benzodiazepine derivatives often induce a drowsy and unconscious state followed by significant difficulty in 'awake ablation', following the operators' instructions is mandatory.

Nagai and colleagues used dexmedetomidine, an alpha-2 adrenergic receptor agonist highly specific to the central nervous system. Its indication has recently been expanded to include nonintubated patients requiring sedation for surgery or short-term procedures. It is also useful as an adjunct for sedation and general anesthesia in certain operations and invasive medical procedures such as colonoscopy. The investigators performed monopolar or bipolar RFA therapy in 23 patients with HCC. The sedative ability of dexmedetomidine was evaluated with six grades using the Ramsay sedation score. During the median ablation period of 14 min, all patients were successfully treated with (n = 18) or without (n = 5) additional analgesic agents. Although Nagai and colleagues added pentazocine or pethidine in some patients, they emphasized that dexmedetomidine was very safe during and after the procedure for elderly patients, and that its administration was easy to control.

Novel Microwave Ablation

Noguchi and colleagues introduced a novel microwave device, the cooled microwave antenna (CMA), which is already available in Western countries. The CMA system has been reported to ablate larger volumes of tissues than previous microwave devices without a tip-cooling system. The investigators compared the ablation ability of the cooled-tip RFA system with that of the CMA device using ex vivo bovine liver. The median size of the ablation area was 46.2 × 34.0 mm after 12 min with the cooled-tip system (n = 5), whereas it was 56.0 × 35.8 mm after 5 min and 69.0 × 48.6 mm after10 min with the CMA system (n = 5). The CMA system thus ablated a larger area in a shorter time, because high ablative power seemed to be provided to a wide range of liver tissues without heating the tip of the electrode. The experts emphasized that the use of the CMA system can result in both a shorter treatment time and a larger ablation volume in the treatment of HCC.

Future Perspective of RFA

The recent development of RFA has expanded the range of treatments of HCC. The main characteristic of RFA therapy is the localized tumor destruction in situ with maximal preservation of the noncancerous part of the liver parenchyma, in contrast to the significant liver damage caused by other interventional therapies such as transcatheter arterial chemoembolization and intra-arterial chemotherapeutic infusion [9].

Future studies from the technical viewpoint should focus on (1) the development of optimal ablation techniques for bipolar and multipolar systems that can generate optimal volumes and shapes of tissue destroyed, (2) varied efforts for reducing side effects (most favorable analgesic therapy, avoidance of biliary tree complication, and so on), (3) various attempts of image assistance to attain more effective RFA procedure, and (4) the assessment of efficacy of multimodal and combined treatments.

Disclosure Statement

The authors declare that no financial or other conflicts of interest exist in relation to the content of this article.

References

1 Kim DY, Han KH: Epidemiology and surveillance of hepatocellular carcinoma. Liver Cancer 2012;1:2–14.
2 Kudo M: Japan's successful model of nationwide hepatocellular carcinoma surveillance highlighting the urgent need for global surveillance. Liver Cancer 2012;1:141–143.
3 Lin SM: Local ablation for hepatocellular carcinoma in Taiwan. Liver Cancer 2013;2:73–83.
4 Mise Y, Sakamoto Y, Ishizawa T, et al: A worldwide survey of the current daily practice in liver surgery. Liver Cancer 2013;2:55–66.
5 Belghiti J, Fuks D: Liver resection and transplantation in hepatocellular carcinoma. Liver Cancer 2012;1:71–82.
6 Lo RC, Ng IO: Hepatic progenitor cells: their role and functional significance in the new classification of primary liver cancers. Liver Cancer 2013;2:84–92.
7 Oishi N, Yamashita T, Kaneko S: Molecular biology of liver cancer stem cells. Liver Cancer 2014;3:71–84.
8 Kawamura Y, Ikeda K, Fukushima T, et al: Potential of a no-touch pincer ablation procedure for small hepatocellular carcinoma that uses a multipolar radiofrequency ablation system: an experimental animal study. Hepatol Res 2013, DOI: 10.1111/hepr.12240.
9 Kudo M: Treatment of advanced hepatocellular carcinoma with emphasis on hepatic arterial infusion chemotherapy and molecular targeted therapy. Liver Cancer 2012;1:62–70.

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Prevention of Disease Progression with Anti-Inflammatory Therapy in Patients with HCV-Related Cirrhosis: A Markov Model

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Key Words
Hepatitis C · Hepatocellular carcinoma · Interferon · Glycyrrhizin · Carcinogenesis · Markov model · Anti-inflammatory therapy

Abstract
Background: The significance of anti-inflammatory therapy has not been fully evaluated in hepatitis C virus (HCV)-related cirrhosis. ***Patients and Methods:*** We analyzed stepwise progression rates from cirrhosis to hepatocellular carcinoma (HCC) and to death using a Markov model in 1,280 patients with HCV-related cirrhosis. During the observation period, 303 patients received interferon and 736 received glycyrrhizin injections as anti-inflammatory therapy. ***Results:*** In the entire group, annual progression rates from cirrhosis to HCC and from cirrhosis to death were 6.8 and 1.9%, and the rate from HCC to death was 19.0%. When sustained virological response (SVR) or biochemical response (BR) was attained with interferon, the annual rate to HCC decreased to 2.6%. On the contrary, the progression rates to HCC and to death in the patients without SVR and BR were 7.2 and 2.0%, respectively ($p < 0.0001$). Continuous interferon administration significantly decreased the carcinogenesis rate to 5.5% ($p = 0.0087$). In the analysis of the remaining patients with high alanine transaminase of 75 IU/l or more but without interferon response or without interferon administration, glycyrrhizin injection significantly decreased annual non-progression probability (no glycyrrhizin 88.0% vs. glycyrrhizin therapy 92.3%, $p = 0.00055$). ***Conclusion:*** Glycyrrhizin injection therapy is useful in the prevention of disease progression in interferon-resistant or intolerant patients with HCV-related cirrhosis.

Introduction

Hepatitis C virus (HCV) is one of the principal etiologies of hepatocellular carcinoma (HCC), with high morbidity and mortality rates in many countries [1–5]. Because interferon has anti-viral, anti-fibrotic and anti-inflammatory properties, it is still a main agent in the treatment of chronic hepatitis C [6, 7]. Many authors have described interferon as capable of preventing hepatocarcinogenesis and prolonging patient survival [8–13]. The radical eradication of HCV by interferon greatly depends on viral load, HCV subtype, certain mutations of the hepatitis virus gene, liver histology, mode of interferon administration and various host factors, including

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the patient's age [10, 13, 14]. When a significant side effect occurs during interferon therapy, cessation or early withdrawal of the therapy often leads to an unsuccessful result. Early withdrawal and treatment failure is usually more common in patients with an advanced stage of liver disease.

Adverse effects of interferon are more commonly found in patients with cirrhosis, and hematological disorders often necessitate cessation of interferon before the therapy is complete. As a result, interferon is considered less effective in the advanced stage of hepatitis. Liver cirrhosis is usually associated with patients aged 55–60 years or older; the adverse effects of interferon-based anti-viral therapy are prevalent in this age group, resulting in low overall compliance for long-term therapy. Because the severity of chronic liver disease is closely associated with the response to interferon therapy [14–16], the sustained response rate is often low in patients with cirrhosis. Furthermore, an older patient with cirrhosis has a very high risk of carcinogenesis and mortality because fibrotic stage is correlated with a patient's age. The role of interferon in suppression of the carcinogenesis rate is therefore likely to be less significant in patients with cirrhosis en masse. There have been several clinical attempts to administer interferon for HCV-related cirrhosis to suppress the hepatocellular carcinogenesis rate [8, 9, 11, 12, 17–19]. However, there have been conflicting reports about the therapeutic value of interferon for this purpose. Some studies have shown a beneficial effect of interferon in reducing carcinogenesis [8, 9, 12, 18], but other reports have not [11, 17, 19].

When interferon fails to eliminate HCV RNA in a patient, long-term administration of interferon often shows anti-carcinogenic action through stabilization of alanine transaminase (ALT) and suppression of the necro-inflammation of hepatocytes [20]. For patients who do not respond to long-term interferon therapy, as shown by persistently high ALT values, glycyrrhizin injection therapy is available in several countries, including some countries in Asia and Europe. A glycyrrhizin-containing product, Stronger Neo-Minophagen C™ (SNMC; Minophagen Pharmaceutical Co. Ltd., Tokyo, Japan), is widely used in Japan for suppression of hepatitis activity and for prevention of disease progression in patients with hepatitis B virus- and HCV-induced chronic hepatitis. Glycyrrhizin has been reported to mitigate hepatic inflammation by suppressing elevated ALT levels and preventing disease progression [21–24]. We previously reported the favorable effects of long-term administration of glycyrrhizin against hepatocellular carcinogenesis in patients with interferon-naïve and interferon-resistant chronic hepatitis C [25, 26].

In order to elucidate whether long-term glycyrrhizin injection therapy suppresses hepatocarcinogenesis and mortality rates in patients with interferon-resistant cirrhosis, we retrospectively analyzed a large cohort of patients with HCV-related cirrhosis in a single institution. The principal aims of our study were to show the clinical role of glycyrrhizin in advanced liver disease, and to determine whether glycyrrhizin can be used as an anti-inflammatory therapy.

Patients and Methods

Study Population and Analyzed Cohorts

A total of 1,358 consecutive patients with hepatitis C were diagnosed as having liver cirrhosis at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan, from 1974 to 2007. They had positive anti-HCV antibody, detectable HCV RNA (nested PCR), and negative hepatitis B surface antigen. Anti-HCV and HCV RNA were assayed using stored frozen sera. Among the 1,358 consecutive patients with hepatitis C, 78 patients were excluded from the study based on meeting one or more of the following exclusion criteria: (1) possible association with HCC; (2) association with hemochromatosis, autoimmune liver disease, primary biliary cirrhosis, α1-antitrypsin deficiency or Wilson disease; (3) daily alcohol ingestion of 75 g or more; (4) α-fetoprotein of 400 ng/ml or higher; (5) a short follow-up period of 6 months or less, or (6) Child-Pugh stage C liver disease because of the substantial difference in carcinogenesis in these patients [27–29].

The remaining 1,280 patients with HCV-positive liver cirrhosis were retrospectively analyzed for hepatocellular carcinogenesis and mortality. Among them, 754 patients (59.4%) were diagnosed as having cirrhosis by histopathological findings with peritoneoscopy and biopsy, and the remaining 526 (40.6%) were diagnosed with clinical findings: rough-surfaced liver on imaging (ultrasonography or computerized tomography, CT), plus endoscopic finding of esophageal varices, overt ascites or indocyanine green retention rate at 15 min of 30% or more. There were 744 men and 536 women, with a median age of 59 years (range 22–86). They were observed for a median of 8.1 years (table 1). A total of 231 patients (18.0%) were lost to follow-up during the observation period.

Interferon Treatment and Evaluation of Effects

Among the 1,280 patients with cirrhosis, 303 patients (23.7%) received interferon therapy with or without ribavirin. Among the 303 patients receiving interferon therapy, 252 received interferon-α and 51 received interferon-β therapy. For dosages, 258 patients received at least 6 million IU/day, and the other 45 patients received no more than 3 million IU/day as their initial anti-viral therapy. Of 303 patients receiving interferon, 52 patients received interferon daily for the first 2–8 weeks and then 2–3 times per week for the following 24–72 weeks. The other 251 patients received interferon 3 times per week for 24–72 weeks. The median administration period was 26.0 weeks (range 4–548).

Table 1. Clinical features of the study group: 1,280 patients with liver cirrhosis caused by hepatitis C

Demography	
Male	744 (58.1)
Female	536 (41.9)
Age, years	59 (22–86)
Decompensated cirrhosis	134 (10.5)
History of blood transfusion	549 (42.9)
Total alcohol intake >500 kg	200 (15.6)
Presence of diabetes mellitus	249 (19.5)
Observation period, years	8.1 (0.5–30.6)
Laboratory data	
ICG R15, %	27 (2–96)
Bilirubin, mg/dl	1.0 (0.2–7.7)
Albumin, g/dl	3.7 (1.6–5.1)
Aspartate transaminase, IU/l	66 (14–1313)
ALT, IU/l	62 (4–570)
Platelet count, $\times 10^3/mm^3$	104 (20–398)
Prothrombin time, %	82 (11–117)
Hepatitis C subtype	
1	821 (75.7)
2	254 (23.4)
Other	9 (0.8)
Treatment after diagnosis of cirrhosis	
Interferon with/without ribavirin	303
Glycyrrhizin injection	736
Ursodeoxycholic acid	615

Data are presented as the median value with range in parentheses, or n with percentages in parentheses. ICG R15 = Indocyanine green retention rate at 15 min.

Almost all patients who received interferon therapy showed varying degrees of influenza-like symptoms, leukocytopenia and thrombocytopenia. Eight patients discontinued interferon therapy because of significant adverse reactions: depression in 2 patients, severe cytopenias in 2, marked anorexia in 1, malaise in 2 and retinopathy in 1 patient. No patients developed decompensated liver disease with ascites, encephalopathy, jaundice or variceal bleeding.

The effects of interferon therapy were classified according to the elimination of HCV RNA and the levels of ALT for 6 months after the end of the treatment. Sustained virological response (SVR) was defined as persistent disappearance of HCV RNA after therapy. Biochemical response (BR) was defined as normal ALT values without elimination of HCV RNA for at least 6 months after therapy. No response (NR) was defined as persistently abnormal or only transient normalization of ALT for a period of less than 6 months. Because 73 patients (24.1%) were still undergoing their course of interferon therapy, the evaluation was conducted in 230 (75.9%) of the 303 patients.

Glycyrrhizin Injection (SNMC) Therapy

Glycyrrhizin therapy was performed using intravenous injections of SNMC™ (Minophagen Pharmaceutical Co. Ltd.). The preparation contains 0.2% (40 mg) glycyrrhizinic acid as the main active constituent, 2% (400 mg) glycine and 0.1% (20 mg) L-cysteine in a 20-ml ampule.

Of 376 chronic hepatitis patients with interferon resistance or who did not receive interferon injection therapy, 264 patients underwent glycyrrhizin injection therapy and the remaining 112 patients did not receive therapy until the end of observation. The purpose of glycyrrhizin injection therapy was to suppress elevated ALT levels and to prevent disease progression in all the patients. In patients for whom the treatment was regarded as effective with respect to ALT levels, the treatment was usually continued for as long a period as possible. As a result, a daily dose of 100 ml of SNMC was administered three times a week for a median period of 4.9 years (range 0.1–24.1) in the glycyrrhizin-treated group.

Certain patients with a high ALT value did not receive glycyrrhizin injection for a variety of reasons. These included the refusal of intravenous treatment, a difficulty in frequently visiting the clinic for the injection, inappropriate superficial veins for repeated injection, negativism towards the handling of intravenous therapy by the doctors in charge, and so on. Those patients who did not receive glycyrrhizin injection therapy in spite of a high ALT often received pills of ursodeoxycholic acid as an anti-inflammatory therapy.

Follow-Up of Patients and Diagnosis of HCC

Follow-up of the patients was made on a monthly to tri-monthly basis after the initial visit. Imaging diagnosis was made one or more times per year with ultrasonography, CT or magnetic resonance imaging. HCC was diagnosed by its typical hypervascular characteristics on CT, magnetic resonance imaging or angiography. When combined use of imaging modes could not demonstrate a typical image of HCC, a fine-needle biopsy was obtained for microscopic examination. The imaging diagnosis was similarly performed among those patients with interferon therapy, glycyrrhizin therapy and without therapy.

Statistical Analysis and Markov Model

Standard statistical measures and procedures were used. The χ^2 test, Fisher exact test and Mann-Whitney U test were used to analyze the differences in demography and laboratory findings. Progression and survival rates were analyzed using the Kaplan-Meier technique [30] with the log-rank test. A Markov model [31] was used to analyze the transition rates from liver cirrhosis to appearance of HCC, and to death. A homogenous Markov chain consisted of three states (fig. 1). These were liver cirrhosis, appearance of HCC and death as an absorbing state from where no transitions to the other states occurred. The model was based on the following principles: (1) the three states are mutually exclusive and collectively exhaustive; (2) the Markov assumption is that the current state has no memories of prior states; (3) the time intervals are uniform, and (4) the transition probabilities are constant and time independent. The first and second items here define a Markov chain, whereas the third and fourth items characterize a homogenous Markov chain [32]. Patient data were regarded as censored at the time of the last date of observation, in the evaluation of survival analysis and Markov analysis.

A p value <0.05 in the two-tailed test was considered significant. Data analysis was performed using IBM SPSS Statistics version 18 [33]. The Human Ethics Review Committee of Toranomon Hospital approved the study protocol.

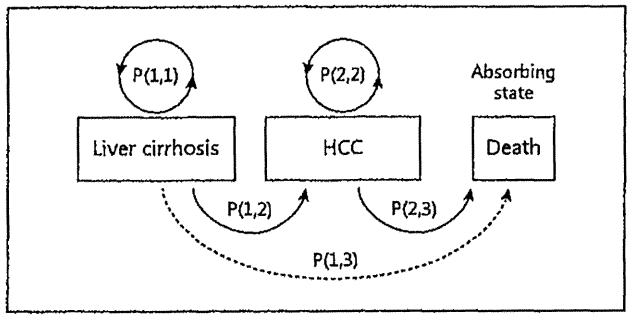

Fig. 1. Markov state transition diagram of liver cirrhosis. Three states were defined: liver cirrhosis without development of HCC, liver cirrhosis-associated HCC, and death. Of these, death was the absorbing state from which no transitions to the other states occurred. The transition in one cycle (1 year) is shown. Arrows connecting two different states indicate observed transitions. The figure represents a probability diagram of the entire study group. All patients were initially at the stage of liver cirrhosis, but transitions to HCC stages gradually increased with time.

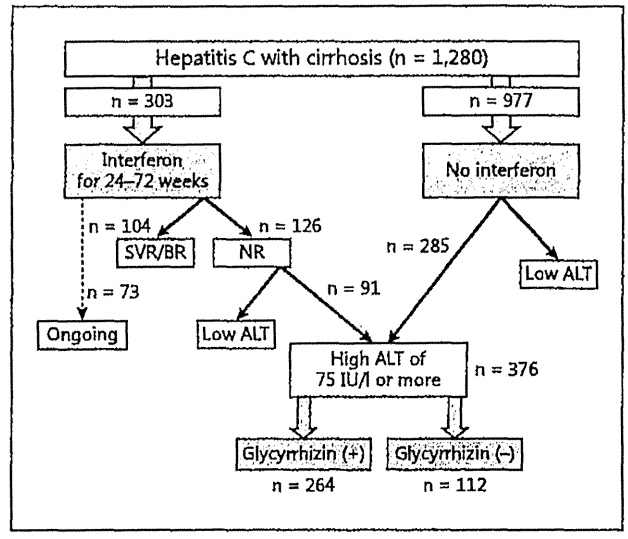

Fig. 2. Clinical courses of patients with cirrhosis. Among 303 patients who received interferon therapy, there were 104 patients who had SVR or BR, and 126 patients who had NR. The remaining 73 patients continued to receive long-term interferon therapy. Among 376 patients with a high ALT value of 75 IU/l or more, with or without a history of interferon therapy, 264 patients underwent glycyrrhizin injection therapy and 112 did not receive glycyrrhizin.

Results

Effects of Interferon and Anti-Inflammatory Treatment

Among the 303 patients who underwent interferon therapy with or without ribavirin, 79 patients (26.1%) showed HCV RNA clearance (SVR effect), and 25 patients (8.3%) showed a BR with normal ALT values for 6 months or longer. One hundred and twenty-six patients (41.6%) showed NR after cessation of interferon. The remaining 73 patients (24.1%) continued intermittent interferon administration for 1 year or longer.

Among the 977 patients who did not receive interferon therapy, plus the 126 patients who received interferon with NR, a high ALT value of 75 IU/l or more was found in 376. Of these patients, 264 (70.2%) underwent long-term glycyrrhizin injection therapy and the other 112 (29.8%) did not receive glycyrrhizin (fig. 2).

Crude Hepatocellular Carcinogenesis and Survival Rates in the Entire Study Group

Cumulative hepatocellular carcinogenesis rates were calculated in all 1,280 study patients with HCV-related cirrhosis. The carcinogenesis rates were 16.4, 29.2, 37.3, 51.6, 65.0 and 69.5% at the end of the third, fifth, seventh, tenth, fifteenth and twentieth years, respectively (fig. 3a). The cumulative survival rates were 93.0, 86.3, 77.1, 61.9, 39.3 and 25.4% at the same time points (fig. 3b).

Probabilities for Transition among the Three Disease States according to the Results of Interferon and Anti-Inflammatory Treatment

In the matrix of the entire study group, 6.8% (562/8,273) of the patients with liver cirrhosis progressed to HCC annually, and 1.9% (157/8,273) died. The remaining 91.3% (7,554/8,273) of the patients remained in the stage of liver cirrhosis after 1 year. Similarly, 19.0% (423/2,228) of the patients in the stage of HCC died, and 81.0% (1,805/2,228) of the patients remained in the stage of HCC annually (table 2).

The results are shown in table 3 as a matrix of transition probabilities for three subsets composed of treatments (SVR or BR, NR or no interferon, and continual interferon) stratified by three states (cirrhosis, HCC, and death). The probabilities for transition from liver cirrhosis to HCC and from liver cirrhosis to death were significantly lower in patients who achieved SVR or BR [2.6% (20/778) and 0.6% (5/778)] than in patients with NR or no interferon therapy [7.2% (542/7,494) and 2.0% (151/7,494); $\chi^2 = 32.4$, $p < 0.0001$]. The probabilities for transition from liver cirrhosis to HCC and from liver cirrhosis to death were significantly lower in patients who

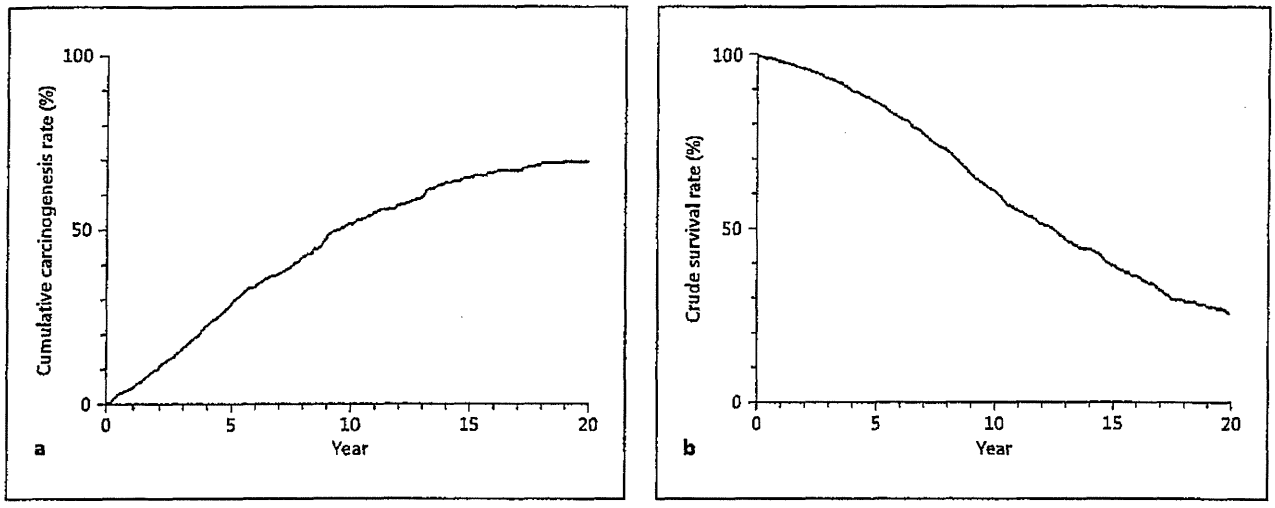

Fig. 3. HCV-positive chronic hepatitis patients with cirrhosis were retrospectively analyzed for hepatocellular carcinogenesis and mortality. **a** Cumulative hepatocellular carcinogenesis rate in the entire group of patients with cirrhosis. **b** Crude survival rate in the entire group of patients with cirrhosis.

Table 2. One-year state-transition probability matrix of the entire study group (n = 10,501 person years)

	Cirrhosis	HCC	Death
Liver cirrhosis (n = 8,273)	7,554 (91.3)	562 (6.8)	157 (1.9)
HCC (n = 2,228)		1,805 (81.0)	423 (19.0)

Figures in parentheses are percentages.

received continuous interferon therapy [5.5% (39/714) and 0.7% (5/714)] than in patients with NR or no interferon therapy [7.2% (542/7,494) and 2.0% (151/7,494); $\chi^2 = 7.59$, $p = 0.0059$].

Probabilities for Transition among the Remaining Patients with High ALT

Among 376 patients without SVR/BR effect and continuous interferon injection and with a high ALT value of 75 IU/l or more, 264 patients (70.2%) received glycyrrhizin injection as anti-inflammatory therapy. Among 692 patients without SVR/BR effect and continuous interferon injection and with relatively low ALT of less than 75 IU/l, glycyrrhizin injection was performed only in 253 patients (36.6%).

We evaluated the transition probabilities among the three states in the remaining patients with high ALT levels of 75 IU/l or more. In the matrix of patients without glycyrrhizin injection therapy, the transition probability from liver cirrhosis to HCC was 6.8% (85/1,245), and the probability of transitioning from cirrhosis to death was 2.0% (25/1,245). In the patients who received glycyrrhizin injection therapy, the transition probability from liver cirrhosis to HCC was 5.9% (45/764), and the probability of transitioning from cirrhosis to death was 0.8% (6/764). Glycyrrhizin injection therapy slightly improved the transition probability both from liver cirrhosis to HCC and from liver cirrhosis to death, but statistical significance was not observed ($\chi^2 = 5.5$, $p = 0.06$; table 4).

Disease Control Rates (Annual Non-Progression Probability) of Anti-Viral and Anti-Inflammatory Treatment

The disease control rates depended on the probabilities for transition between progression and non-progression of disease at a specific time interval, which was set at 1 year. The yearly transition probabilities were calculated based on the data of 10,501 person years of the 1,280 study patients with HCV-positive liver cirrhosis.

The disease control rate of the patients with SVR or BR (874/910, 96.0%) was significantly higher than that of the

Table 3. One-year state-transition probability matrices according to initial treatment

	Cirrhosis	HCC	Death
Patients with SVR or BR (n = 910 person years)			
Liver cirrhosis (n = 778)	753 (96.8)	20 (2.6)	5 (0.6)
HCC (n = 132)		121 (91.7)	11 (8.3)
Patients with no response or no interferon therapy (n = 9,590 person years)			
Liver cirrhosis (n = 7,494)	6,801 (90.8)	542 (7.2)	151 (2.0)
HCC (n = 2,096)		1,684 (80.3)	412 (19.7)
Patients with continuous interferon therapy (n = 856 person years)			
Liver cirrhosis (n = 714)	670 (93.8)	39 (5.5)	5 (0.7)
HCC (n = 142)		132 (93.0)	10 (7.0)

Figures in parentheses are percentages.

Table 4. One-year state-transition probability matrices according to glycyrrhizin injection therapy for patients with high ALT values

	Cirrhosis	HCC	Death
Patients without glycyrrhizin therapy (n = 1,637 person years)			
Liver cirrhosis (n = 1,245)	1,135 (91.2)	85 (6.8)	25 (2.0)
HCC (n = 392)		305 (77.8)	87 (22.2)
Patients with glycyrrhizin therapy (n = 913 person years)			
Liver cirrhosis (n = 764)	713 (93.3)	45 (5.9)	6 (0.8)
HCC (n = 149)		130 (87.2)	19 (12.8)

Figures in parentheses are percentages.

Table 5. One-year non-progression probability matrix of anti-viral and anti-inflammatory treatment

	Non-progression	Progression
Entire study group (n = 10,501)	9,359 (89.1)	1,142 (10.9)
Patients with SVR or BR (n = 910)	874 (96.0)	36 (4.0)
Patients with NR or no interferon therapy (n = 9,590)	8,485 (88.5)	1,105 (11.5)
Patients without glycyrrhizin therapy (n = 1,637)	1,440 (88.0)	197 (12.0)
Patients with glycyrrhizin therapy (n = 913)	843 (92.3)	70 (7.7)

Figures in parentheses are percentages.

patients with NR or the patients without interferon therapy (8485/9590, 88.5%; $\chi^2 = 49.1$, $p < 0.0001$).

We also evaluated disease control rates according to glycyrrhizin injection therapy in the subgroups of patients who either failed or did not receive interferon therapy with a high ALT of 75 IU/l or more. Anti-inflammation therapy with glycyrrhizin injections significantly increased the disease control rates, as shown by the rate of 92.3% (843/913) in the patients who received glycyrrhizin injection therapy versus 88.0% (1,440/1,637) in the patients without glycyrrhizin therapy ($\chi^2 = 11.9$, $p < 0.0001$; table 5).

Discussion

Based on our epidemiological data obtained from long-term observations of patients with chronic hepatitis [34] and patients with cirrhosis [35], we found that the life expectancy of patients with HCV-related liver cirrhosis heavily depends on the development of HCC. The probability of patients with HCV-related liver cirrhosis eventually developing HCC is staggeringly high at 75% [35]. In the present study, interferon administration significantly decreased the probability for transition from liver cirrhosis to HCC in the patients who achieved SVR or BR. However, there were some background varieties between the patients with SVR or BR and NR or no interferon therapy with respect to stage of fibrosis, sex, platelet count and age, which can affect the carcinogenesis rate.

From the standpoint of anti-inflammatory effects and cancer prevention [8–10, 13, 14, 19], interferon is effective in patients with chronic liver disease caused by HCV. Although the carcinogenesis rate is noticeably reduced when the ALT level becomes normal with or without HCV RNA eradication [10, 13, 14] after therapy, ALT levels become normal after interferon therapy in approximately half of the patients with a high viral load and group 1 HCV subtype. Furthermore, the anti-carcinogenic capacity of interferon has been demonstrated not only in patients with persistent ALT normalization, but also in patients with transient normalization of ALT for at least 6 or 12 months [20].

Many authors have already described that the activity of interferon in suppressing the development of HCC in patients with HCV RNA clearance (SVR) is similar to that in patients with ALT normalization in the absence of elimination of HCV RNA (BR) [13, 36–38]. Based on these compelling lines of evidence, the anti-carcinogenic activity of interferon is ascribed to the suppression of in-

flammatory and regenerative processes in hepatocytes. Moreno and Muriel [39] reported that interferon reverses liver fibrosis and, therefore, control of the necro-inflammatory process can suppress the growth of HCC.

An SVR improves clinical symptoms in decompensated cirrhosis [40], but interferon often induces severe complications, even in young patients with decompensated cirrhosis [41]. A patient with compensated cirrhosis can be a candidate for interferon therapy if careful, close hematologic monitoring is performed.

Because patients with liver cirrhosis generally experience some difficulties with interferon treatment, our present study demonstrated practical information about carcinogenesis and the life expectancy of patients with HCV-related liver cirrhosis and the order of priority in the management of interferon for these patients. Interferon administration is considered and initiated in patients with HCV-related liver cirrhosis preferably to reduce the probability for the transition from liver cirrhosis to HCC.

Because carcinogenesis is not a single-step event, but rather a complex, multi-step process, the exact mechanism of the role of glycyrrhizin in suppressing liver carcinogenesis remains unknown. One of the principal functions of long-term administration of glycyrrhizin in decreasing the carcinogenesis rate is considered to be anti-inflammation, which blocks the active carcinogenic process of continuous hepatic necro-inflammation and cell damage. In the treated group of the present study, the median ALT values markedly decreased after initiation of the glycyrrhizin injections, suggesting that the pathological process of hepatocyte necrosis or apoptosis was significantly suppressed by glycyrrhizinic acid. The actions of the amino acids, glycine and cysteine contained in SNMC have not been completely explained, but these substances have been demonstrated to suppress increased aldosterone levels that are induced by glycyrrhizinic acid. Tarao et al. [42] reported that a high ALT level resulted in an increased HCC recurrence rate in patients with HCC. From the standpoint of these anti-inflammatory activities, SNMC may be considered to only postpone the time of HCC appearance in the clinical course of cirrhosis. Since the entire process of hepatocellular carcinogenesis from the initial transformation of a hepatocyte to a detectable growth of cancer is considered to take at least several years, the influence of glycyrrhizin on the carcinogenesis rate cannot be evaluated over a short period.

Because the data in the present study were obtained from a retrospective cohort analysis, glycyrrhizin doses, times of injection per week and duration of therapy varied in each patient in the treated group. In order to elucidate the cancer preventive effect of glycyrrhizin therapy in active HCV-related liver disease, we should further stratify the treated patients or perform much more detailed statistical procedures. Future studies should aim at defining the basic oncogenic mechanisms and roles of long-term administration of glycyrrhizin in carcinogenesis in chronic hepatitis patients with cirrhosis caused by HCV.

In conclusion, the results of the present study demonstrated that long-term intermittent glycyrrhizin (SNMC) therapy for a few years or more successfully reduced disease progression probability (progression to carcinogenesis plus progression to death) in patients with HCV-related cirrhosis. A randomized controlled trial with a larger number of cases, with or without glycyrrhizin therapy, is expected to confirm the effectiveness of this therapy.

References

1 Bruix J, Barrera JM, Calvet X, Ercilla G, Costa J, Sanchez-Tapias JM, Ventura M, Vall M, Bruguera M, Bru C, et al: Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. Lancet 1989;2:1004–1006.

2 Colombo M, Kuo G, Choo QL, Donato MF, Del Ninno E, Tommasini MA, Dioguardi N, Houghton M: Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. Lancet 1989;2:1006–1008.

3 Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, Kumada H: A multivariate analysis of risk factors for hepatocellular carcinogenesis – a prospective observation of 795 cases with viral and alcoholic cirrhosis. Hepatology 1993;18:47–53.

4 Williams I: Epidemiology of hepatitis C in the United States. Am J Med 1999;107:2S–9S.

5 Alter MJ: Epidemiology of hepatitis C virus infection. World J Gastroenterol 2007;13:2436–2441.

6 Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC, Perrillo RP, Carey W, Jacobson IM, Payne J, Dienstag JL, et al: Treatment of chronic hepatitis C with recombinant interferon alfa: a multicenter, randomized, controlled trial. N Engl J Med 1989;321:1501–1506.

7 Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, Goodman Z, Banks SM, Hoofnagle JH: Recombinant interferon alfa therapy for chronic hepatitis C: A randomized, double-blind, placebo-controlled trial. N Engl J Med 1989;321:1506–1510.

8 Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, Shiomi S, Seki S, Kobayashi K, Otani S: Randomized trial of effects of interferon-α on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 1995;346:1051–1055.

9 Mazzella G, Accogli E, Sottili S, Festi D, Orsini M, Salzetta A, Novelli V, Cipolla A, Fabbri C, Pezzoli A, Roda E: Alpha interferon treatment may prevent hepatocellular carcinoma in HCV-related liver cirrhosis. J Hepatol 1996;24:141–147.

10 Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, Iijima A, Urushihara A, Kiyosawa K, Okuda M, Hino K, Okita K: Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C: Osaka Liver Disease Study Group. Hepatology 1998;27:1394–1402.
11 Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hurter D: Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 1998;28:1687–1695.
12 International Interferon-α Hepatocellular Carcinoma Study Group: Effect of interferon-α on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. Lancet 1998;351:1535–1539.
13 Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi, Tsubota A, Nakamura I, Murashima N, Kumada H, Kawanishi M: Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology 1999;29:1124–1130.
14 Yabu K, Kiyosawa K, Mori H, Matsumoto A, Yoshizawa K, Tanaka E, Furuta S: Serum collagen type IV for the assessment of fibrosis and resistance to interferon therapy in chronic hepatitis C. Scand J Gastroenterol 1994;29:474–479.
15 Yoshioka K, Kakumu S, Hayashi H, Shinagawa T, Wakita T, Ishikawa T, Itoh Y, Takayanagi M: Anti-hepatitis C antibodies in patients with chronic non-A, non-B hepatitis: relation to disease progression and effect of interferon alpha. Am J Gastroenterol 1991;86:1495–1499.
16 Tsubota A, Chayama K, Ikeda K, Arase Y, Koida I, Saitoh S, Hashimoto M, Iwasaki S, Kobayashi M, Hiromitsu K: Factors predictive of response to interferon-α therapy in hepatitis C virus infection. Hepatology 1994;19:1088–1094.
17 Schalm SW, Fattovich G, Brouwer JT: Therapy of hepatitis C: patients with cirrhosis. Hepatology 1997;26 (suppl):S128–S132.
18 Benvegnu L, Chemello L, Noventa F, Fattovich G, Pontisso P, Alberti A: Retrospective analysis of the effect of interferon therapy on the clinical outcome of patients with viral cirrhosis. Cancer 1998;83:901–909.
19 Hu KQ, Tong MJ: The long-term outcomes of patients with compensated hepatitis C virus-related cirrhosis and history of parenteral exposure in the United States. Hepatology 1999;29:1311–1316.
20 Ikeda K, Arase Y, Saitoh S, Kobayashi M, Someya T, Hosaka T, Sezaki H, Akuta N, Suzuki F, Suzuki Y, Kumada H: Anticarcinogenic impact of interferon on patients with chronic hepatitis C: a large-scale long-term study in a single center. Intervirology 2006;49:82–90.
21 Fujisawa K, Watanabe Y, Kimura K: Therapeutic approach to chronic active hepatitis with glycyrrhizin. Asian Med J 1980:23:745–756.
22 Suzuki H, Ohta Y, Takino T: Effects of glycyrrhizin on biochemical tests in patients with chronic hepatitis: double blind trial. Asian Med J 1983;26:423–438.
23 Wildhirt E: Experience in Germany with glycyrrhizinic acid for the treatment of chronic viral hepatitis. Viral Hepat Liver Dis 1994;658–661.
24 van Rossum TGJ, Vulto AG, Hop WCJ, Brouwer JT, Niesters HG, Schalm SW: Intravenous glycyrrhizin for the treatment of chronic hepatitis C: a double-blind, randomized, placebo-controlled phase I/II trial. J Gastroenterol Hepatol 1999;14:1093–1099.
25 Arase Y, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I, Suzuki Y, Saitoh S, Kobayashi M, Kumada H: The long-term efficacy of glycyrrhizin in chronic hepatitis C patients. Cancer 1997;79:1494–1500.
26 Ikeda K, Arase Y, Kobayashi M, Saitoh S, Someya T, Hosaka T, Sezaki H, Akuta N, Suzuki Y, Suzuki F, Kumada H: A long-term glycyrrhizin injection therapy reduces hepatocellular carcinogenesis rate in patients with interferon-resistant active chronic hepatitis C: a cohort study of 1,249 patients. Dig Dis Sci 2006;51:603–609.
27 Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R: Transection of the esophagus in bleeding oesophageal varices. Br J Surg 1973;60:648–652.
28 Tsai JF, Jeng JE, Ho MS, Chang WY, Hsieh MY, Lin ZY, et al: Effect of hepatitis C and B virus infection on risk of hepatocellular carcinoma: a prospective study. Br J Cancer 1997;76:968–974.
29 Bolondi L, Sofia S, Siringo S, Gaiani S, Casali A, Zironi G, Piscaglia F, Gramantieri L, Zanetti M, Sherman M: Surveillance programme of cirrhotic patients for early diagnosis and treatment of hepatocellular carcinoma: a cost effectiveness analysis. Gut 2001;48:251–259.
30 Kaplan EL, Meier P: Nonparametric estimation for incomplete observation. Jam Stat Assoc 1958;53:457–481.
31 Beck JR, Pauker SG: The Markov process in medical prognosis. Med Decis Making 1983;3:419–458.
32 Silverstein MD, Albert DA, Hadler NM, Ropes MW: Prognosis of SLE: comparison of Markov model to life table analysis. Clin Epidemiol 1988;41:623–633.
33 IBM SPSS: IBM SPSS for Windows version 18.0 manual. Armonk, SPSS Japan Inc., 2009.
34 Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, Arase Y, Fukuda M, Chayama K, Murashima N, Kumada H: Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2,215 patients. J Hepatol 1998;28:930–938.
35 Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, Kumada H, Kawanishi M: A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. Hepatology 1993;18:47–53.
36 Kasahara A, Hayashi N, Mochizuki K, Hiramatsu N, Sasaki Y, Kakumu S, Kiyosawa K, Okita K: Clinical characteristics of patients with chronic hepatitis C showing biochemical remission, without hepatitis C virus eradication, as a result of interferon therapy. The Osaka Liver Disease Study Group. J Viral Hepat 2000;7:343–351.
37 Yabuuchi I, Imai Y, Kawata S, Tamura S, Noda S, Inada M, Maeda Y, Shirai Y, Fukuzaki T, Kaji I, Ishikawa H, Matsuda Y, Nishikawa M, Seki K, Matsuzawa Y: Long-term responders without eradication of hepatitis C virus after interferon therapy: characterization of clinical profiles and incidence of hepatocellular carcinoma. Liver 2000;20:290–295.
38 Okanoue T, Itoh Y, Minami M, Sakamoto S, Yasui K, Sakamoto M, Nishioji K, Murakami Y, Kashima K: Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1,148 patients. J Hepatol 1999;30:653–659.
39 Moreno MG, Muriel P: Remission of liver fibrosis by interferon-α 2b. Biochem Pharmacol 1995;50:515–520.
40 Iacobellis A, Siciliano M, Perri F, Annicchiarico BE, Leandro G, Caruso N, Accadia L, Bombardieri G, Andriulli A: Peginterferon α-2b and ribavirin in patients with hepatitis C virus and decompensated cirrhosis: a controlled study. J Hepatol 2007;46:206–212.
41 Nevens F, Goubau P, Van Eyken P, Desmyter J, Desmet V, Fevery J: Treatment of decompensated viral hepatitis B-induced cirrhosis with low doses of interferon alpha. Liver 1993;13:15–19.
42 Tarao K, Takemiya S, Tamai S, Sugimara Y, Ohkawa S, Akaike M, Tanabe H, Shimizu A, Yoshida M, Kakita A: Relationship between the recurrence of hepatocellular carcinoma (HCC) and serum alanine aminotransferase levels in hepatectomized patients with hepatitis C virus-associated cirrhosis and HCC. Cancer 1997;79:688–694.

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Correlation Between Hepatitis B Virus Surface Antigen Level and Alpha-Fetoprotein in Patients Free of Hepatocellular Carcinoma or Severe Hepatitis

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Alfa-fetoprotein (AFP) is used as a marker of early hepatocarcinogenesis. However, the impact of hepatitis B virus surface antigen (HBsAg) on this relationship in patients with HBV infection is not clear. The present study evaluated the relation between HBsAg and AFP levels at the initial visit in 1,610 untreated HBV patients, free of hepatocellular carcinoma (HCC) or severe hepatitis. The cumulative rate of HCC was significantly lower in patients with a low AFP level (≤10 μg/L; below the upper limit of normal) than in those with a high AFP level (≥11 μg/L) at the initial visit. In patients with HBsAg levels more than 500 IU/ml, HBsAg levels correlated significantly and negatively with AFP levels, and significantly with platelet count. Multivariate analysis of data of patients with HBsAg more than 500 IU/ml identified HBsAg (<7,000 IU/ml), albumin (<3.9 g/dl), platelet count ($<20.0 \times 10^4/mm^3$), gamma-glutamyl transpeptidase (≥50 IU/L), aspartate aminotransferase (≥34 IU/L), HBeAg (positive), and HBV core-related antigen (≥3.0 log U/ml) as determinants of a high AFP. Especially, in patients with HBsAg more than 500 IU/ml and low transaminase levels (below the upper limit of normal), HBsAg was identified as significant determinant of a high AFP. On the other hand, in patients with HBsAg less than 500 IU/ml, multivariate analysis identified albumin, gamma-glutamyl transpeptidase, and HBV core-related antigen as determinants of a high AFP. The results indicated that HBsAg level seems to affect, at least in part, the AFP levels, and that it can be used as a surrogate marker of early hepatocarcinogenesis. ***J. Med. Virol. 86:131–138, 2014.*** © 2013 Wiley Periodicals, Inc.

KEY WORDS: HBV; AFP; HBsAg; HBcrAg; genotype; hepatocellular carcinoma

INTRODUCTION

Hepatitis B virus (HBV) is a small, enveloped DNA virus known to cause chronic hepatitis and often leads to liver cirrhosis and hepatocellular carcinoma (HCC) [Viola et al., 1981; Kobayashi et al., 2002; Yao, 2003]. Evidence suggests that the use of elevated alpha-fetoprotein (AFP) for the prediction of early hepatocarcinogenesis in non-HCC patients could be clinically useful. AFP is a fetal glycoprotein produced by the yolk sac and fetal liver [Bergstrand and Czar, 1956] and has been widely used as a serum marker for the diagnosis of HCC [Sato et al., 1993; Johnson, 2001]. Furthermore, high serum AFP levels are also associated with various chronic liver diseases and hepatic regeneration [Kew et al., 1973; Silver et al., 1974; Elftherious et al., 1977; Alpert and Feller, 1978]. Many patients with chronic hepatitis B who are free of HCC have high AFP levels [Chen and Sung, 1979; Di Bisceglie and Hoofnagle, 1989], and some patients with cirrhosis and concomitant high

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inflammatory activity have very high AFP levels [Yao, 2003; Cheema et al., 2004]. On the other hand, some patients with small HCC lesions have only moderately elevated levels of AFP [Shinagawa et al., 1984; Ebara et al., 1986; Bruix and Sherman, 2005]. At present, however, there are no cutoff levels for serum AFP used to predict HCC in patients with HBV infection.

There is growing interest in the use of hepatitis B surface antigen (HBsAg) level as a prognostic marker in chronic hepatitis B patients [Chan et al., 2010]. The HBsAg levels are useful for identifying the stage of disease [Jaroszewicz et al., 2010; Nguyen et al., 2010], to distinguish true inactive carriers from patients with HBe antigen-negative disease [Brunetto et al., 2010; Martinot-Peignoux et al., 2010; Chan et al., 2011; Liaw, 2011], and to predict the response to interferon therapy [Brunetto et al., 2009; Moucari et al., 2009]. Recent studies has also demonstrated that the HBsAg levels are associated with the risk of progression to HCC, especially in patients with low HBV DNA levels [Chan, 2012; Tseng et al., 2012], and that there is a potential correlation between the HBsAg levels and the stage of liver fibrosis [Seto et al., 2012; Martinot-Peignoux et al., 2013]. However, the impact of viral factors, such as the HBsAg level, on serum AFP level as a predictor of early HCC is not clear at present.

The present study included 1,610 untreated patients with HBV infection, free of HCC or severe hepatitis. The present study was designed to provide answers to the following questions: (1) what is the relation between a high serum AFP level at the initial outpatient visit and subsequent development of hepatocarcinogenesis in antiviral-therapy-naive patients with hepatitis B viral infection? (2) What is the impact of viral factors, such as the HBsAg level, on serum AFP level in such patients, and (3) What is a good surrogate marker for a high serum AFP at the initial visit.

PATIENTS AND METHODS

Patients

Among 6,466 consecutive patients who were diagnosed with HBV infection between March 1972 and December 2012 at Toranomon Hospital, 1,610 were selected in the present study based on the following criteria: (1) They were positive for HBsAg (radioimmunoassay, Dainabot, Tokyo, Japan) and negative for anti-HCV (third-generation enzyme immunoassay, Chiron, CA). (2) They were free of HCC at the initial visit. (3) HBV hepatitis was assessed as less than severe at the initial visit, in order to minimize the potential effects of high inflammatory activity. Severe hepatitis was defined as serum transaminase level of ≥300 IU/L, and/or total bilirubin level of ≥3.0 mg/dl. (4) They had not received antiviral therapy in the past (e.g., interferon and/or nucleot(s)ide analogs) at the initial visit. (5) They underwent examination of the AFP level (upper limit of normal, 10 μg/L) at the initial visit. Furthermore, the HBsAg level, HBV core-related antigen (HBcrAg) level, and HBV DNA were also assayed using stored frozen sera obtained at the initial visit. (6) They were free of coinfection with human immunodeficiency virus. (7) They were free of other types of chronic liver disease, including hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, autoimmune liver disease, inherited liver disease including alpha-1 antitrypsin deficiency, and hepatic venous outflow block. (8) They consented to the study.

Table I summarizes the profile and laboratory data at the initial visit of the 1,610 patients included in the present study. They included 1,047 males and 563 females, with a median age of 40 years (range: 18–83 years). The median AFP level was 4 μg/L (range, 1–1,770 μg/L) and the median follow-up time (from the initial visit until the last visit) was 6.0 years (range, 0.0–34.6 years). The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital.

Laboratory Tests

HBsAg, HBcrAg, and HBV DNA levels were assayed using stored frozen sera obtained at the initial visit. Blood samples were frozen at −80°C within 4 hr of collection and were not thawed until used for testing. Serum HBsAg level was measured using Architect HBsAg QT assay kit (Abbott Laboratories, Tokyo, Japan), which has a lower limit of detection of

TABLE I. Profiles and Laboratory Data at the Initial Visit of 1,610 Patients Infected With HBV

Demographic data	
Number of patients	1,610
Sex (male/female)	1,047/563
Age (years)*	40 (18–83)
Family history of liver disease[a]	836 (51.9%)
Lifetime cumulative alcohol intake (≥500 kg)	112 (7.0%)
Laboratory data*	
Total bilirubin (mg/dl)	0.6 (0.1–2.9)
Aspartate aminotransferase (IU/L)	37 (5–220)
Alanine aminotransferase (IU/L)	48 (5–297)
Albumin (g/dl)	4.2 (1.0–5.6)
Gamma-glutamyl transpeptidase (IU/L)	37 (2–2,370)
Hemoglobin (g/dl)	14.5 (6.9–18.2)
Platelet count (×104/mm^3)	19.1 (2.7–44.7)
Alpha-fetoprotein (μg/L)	4 (1–1,770)
Virological data	
HBeAg (No. of positive)	690 (42.9%)
HBsAg (IU/ml)*	2,845 (0.09 to >125,000)
HBcrAg (log U/ml)*	4.9 (<3.0 to >6.8)
HBV DNA (log copies/ml)*	5.7 (<2.1 to >9.1)
HBV genotype (A/B/C/others/ND)	65/218/1,119/6/202

Data are number and percentages of patients, except those denoted by *, which represent the median (range) values.
[a]Family history of positivity for hepatitis B surface antigen including third-degree relatives.

0.05 IU/ml and upper limit of detection of 250 IU/ml. To expand the upper range from 250 to 125,000 IU/ml, serum samples with the HBsAg levels above the upper range were diluted in a stepwise fashion to 1:20 and 1:500 with Architect diluents using the information supplied by the manufacturer. HBeAg was determined by enzyme-linked immunosorbent assay kit (HBeAg EIA; Institute of Immunology, Tokyo, Japan). Serum HBcrAg level was measured using a Cleia HBcrAg assay kit (Fujirebio, Tokyo, Japan) using a fully automated analyzer system (Lumipulse System; Fujirebio). The cut-off value of HBcrAg was 3.0 log U/ml. HBV DNA was quantified using the Cobas TaqMan HBV v.2.0 (Roche Diagnostics, Tokyo, Japan), which has a dynamic range of 2.1–9.0 log copies/ml.

A commercial kit (HBV Genotype EIA; Institute of Immunology) was used to determine serologically the HBV genotypes using the combination of epitopes expressed on the pre-S2 region product, which is specific to each of the major genotypes.

Follow-Up and Diagnosis of Future Hepatocellular Carcinoma

After the initial visit, patients were followed-up once or three times a month. Imaging studies (ultrasonography, computed tomography, or magnetic resonance imaging) were conducted once or more per year.

Statistical Analysis

Non-parametric tests (Mann–Whitney *U*-test, chi-squared test and Fisher's exact probability test) were used to compare differences between two groups. Correlation analysis was evaluated by the Spearman rank correlation test. The cumulative rate of hepatocarcinogenesis was calculated using the Kaplan–Meier technique; differences between cumulative carcinogenesis curves between groups were tested using the log-rank test. Statistical analyses of the rate of hepatocarcinogenesis according to groups were calculated using the period from the initial visit. Univariate and multivariate logistic regression analyses were used to determine the independent surrogate markers of elevated AFP at the initial visit. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. A two-tailed *P*-value less than 0.05 was considered significant. Variables that achieved statistical significance ($P < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors for elevated AFP. Potential surrogate markers of elevated AFP at the initial visit included the following pretreatment variables: age, sex, family history of liver disease, lifetime cumulative alcohol intake, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (GGT), hemoglobin, platelet count, HBV genotype, HBeAg, HBsAg levels, HBcrAg levels, and HBV DNA levels. Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL).

RESULTS

Cumulative Rate of Hepatocarcinogenesis According to the AFP Level at the Initial Visit

A total of 1,061 patients naïve to antiviral therapy from the initial visit until the last visit were evaluated for the rate of development of HCC based on the AFP levels at the initial visit. During the follow-up period, HCC was diagnosed in 31 of 905 patients (3.4%) with a low AFP level (≤10 μg/L; below the upper limit of normal) and 37 of 156 patients (23.7%) with a high AFP level (≥11 μg/L) at the initial visit. The cumulative hepatocarcinogenesis rates for patients with low and high AFP levels at the initial visit were 4.7% and 30.2% at the end of 10-year follow-up; 9.1% and 36.5% at the end of 20-year follow-up; and 13.2% and 42.9% at the end of 30-year follow-up, respectively. These results indicate that the rate of hepatocarcinogenesis is significantly higher in patients with HBV infection and high AFP levels than their counterparts with low AFP levels ($P < 0.001$; Log-rank test) (Fig. 1).

HBsAg and AFP Levels at the Initial Visit

Blood samples from all patients were analyzed to determine the relationship between the HBsAg and the AFP levels at the initial visit. The proportions of patients with high AFP levels among those with the HBsAg levels below 500 IU/ml, from 500 to 1,999 IU/ml, from 2,000 to 6,999 IU/ml, from 7,000 to 24,999 IU/ml, and above 25,000 IU/ml were 12.6% (42 of 333 patients), 26.7% (89 of 333), 22.6% (94 of 416), 10.4% (29 of 278), and 6.4% (16 of 250), respectively (Fig. 2A). The relationship between the HBsAg and

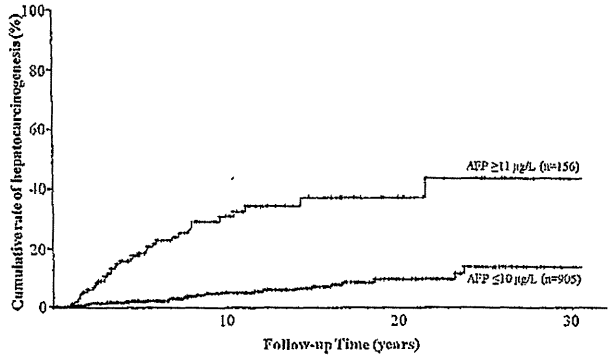

Fig. 1. Cumulative rate of hepatocarcinogenesis according to the AFP level at the initial visit in patients naïve to antiviral therapy from the initial visit until the last visit. The rate of hepatocarcinogenesis was significantly higher in patients with high AFP levels (≥11 μg/L) than in those with low levels (≤10 μg/L) at the initial visit ($P < 0.001$; Log-rank test).

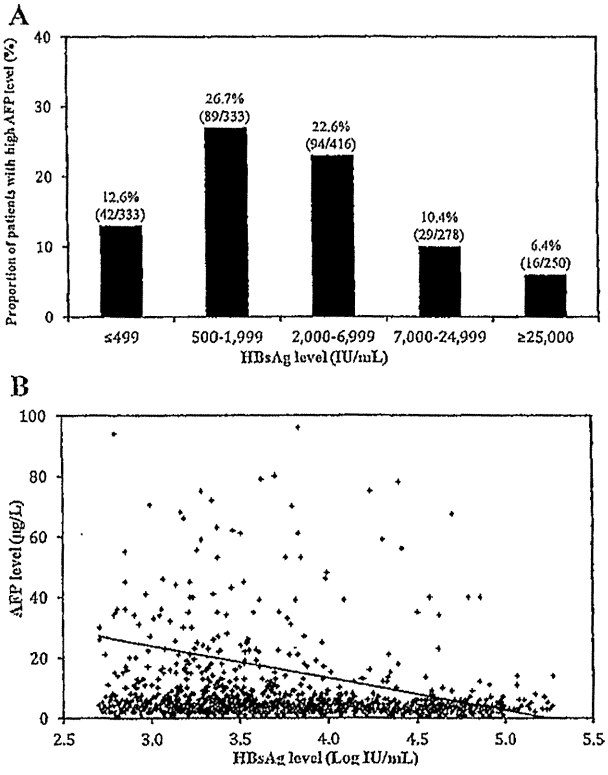

Fig. 2. **A**: Proportions of patients with the high AFP levels (≥11 μg/L) at the initial visit, stratified according to the HBsAg levels. Patients with the HBsAg levels above 500 IU/ml included a significantly lower proportions of patients with the high AFP levels and the HBsAg levels above 7,000 IU/ml (8.5%) than those with the HBsAg levels below 7,000 IU/ml (24.4%) ($P < 0.001$). **B**: Analysis of data of patients with the HBsAg levels above 500 IU/ml at the initial visit, showed a significant negative correlation between logarithmically transformed HBsAg and AFP levels ($r = -0.225$, $P < 0.001$).

the AFP levels at the initial visit suggested the presence of two distinct populations within the study group. Especially, in 1,277 patients with the HBsAg levels above 500 IU/ml, a significantly smaller proportion of patients with high AFP levels were noted among those with HBsAg of more than 7,000 IU/ml (8.5%) than those with the HBsAg levels less than 7,000 IU/ml (24.4%) ($P < 0.001$). Furthermore, the HBsAg levels correlated negatively but significantly with the AFP levels ($r = -0.225$, $P < 0.001$) (Fig. 2B).

The HBsAg Levels and the Platelet Count at the Initial Visit

Blood samples from all patients were analyzed to determine the relationship between the HBsAg levels and the platelet count at the initial visit. The median platelet counts among patients with the HBsAg levels below 500 IU/ml, from 500 to 1,999 IU/ml, from 2,000 to 6,999 IU/ml, from 7,000 to 24,999 IU/ml, and above 25,000 IU/ml were $19.1 \times 10^4/mm^3$, $17.2 \times 10^4/mm^3$, $18.0 \times 10^4/mm^3$, $20.9 \times 10^4/mm^3$, and $21.2 \times 10^4/mm^3$, respectively (Fig. 3A). The relationship between the HBsAg levels and the platelet count at the initial visit also suggested the presence of two distinct populations within the study group. Especially, in 1,277 patients with the HBsAg levels of more than 500 IU/ml, significantly higher platelet counts were noted among those with the HBsAg levels of more than 7,000 IU/ml (the median platelet count; $21.0 \times 10^4/mm^3$) than those with the HBsAg levels less than 7,000 IU/ml (the median platelet count; $17.6 \times 10^4/mm^3$) ($P < 0.001$). Furthermore, the HBsAg

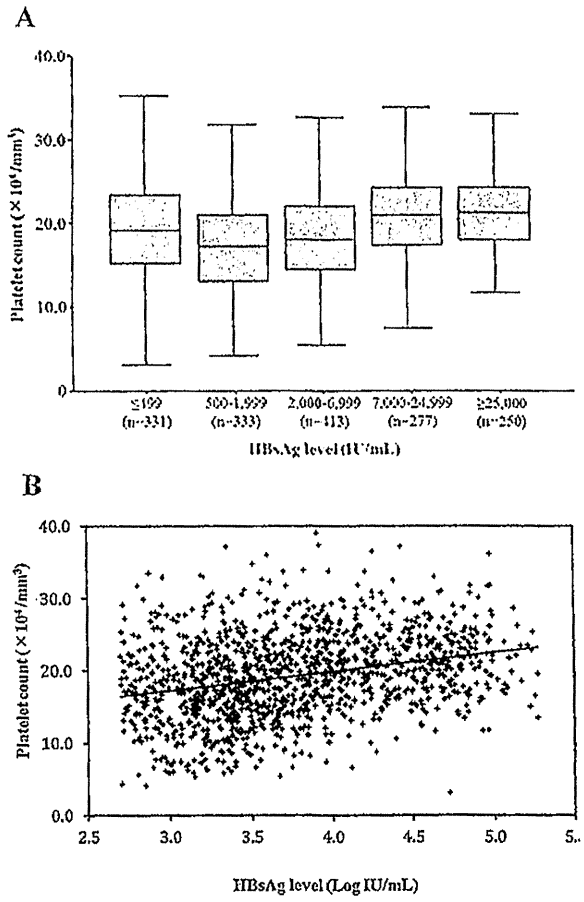

Fig. 3. **A**: The platelet count at the initial visit, stratified according to the HBsAg levels. Bars within the boxes indicate the median platelet count. The boxes denote the 25th to 75th percentiles, the lower and upper bars the 10th and 90th percentiles, respectively. Among patients with the HBsAg levels above 500 IU/ml at the initial visit, those with the HBsAg levels above 7,000 IU/ml had significantly higher platelet count (the median platelet count; $21.0 \times 10^4/mm^3$) compared to those with the HBsAg levels below 7,000 IU/ml (the median platelet count; $17.6 \times 10^4/mm^3$) ($P < 0.001$). **B**: Among patients with the HBsAg levels above 500 IU/ml at the initial visit, logarithmically transformed the HBsAg levels correlated significantly with the platelet count ($r = 0.293$, $P < 0.001$).

levels correlated significantly and positively with the platelet count ($r = 0.293$, $P < 0.001$) (Fig. 3B).

Clinical Profiles and Laboratory Data According to the HBsAg Level at the Initial Visit

Table II summarizes the clinical profiles and laboratory data according to the HBsAg level at the initial visit of 1,610 patients infected with HBV. Patients with the HBsAg levels below 500 IU/ml were significantly older and exhibited lower inflammatory activity (lower levels of AST and ALT), and had lower viral levels (they were HBeAg negative and had lower levels of HBcrAg/HBV DNA), compared to those with the HBsAg levels above 500 IU/ml ($P < 0.001$).

Factors Associated With High AFP Levels at the Initial Visit, Stratified According to the HBsAg Levels

Blood samples from all 1,610 patients were analyzed to determine the factors that affect the AFP level at the initial visit. Among 1,277 patients with the HBsAg levels more than 500 IU/ml at the initial visit, high AFP levels were detected in 228 (17.9%) patients. Univariate analysis identified 12 parameters that correlated significantly with a high AFP level at the initial visit. These included age (≥30 years; $P < 0.001$), AST (≥34 IU/L; $P < 0.001$), ALT (≥43 IU/L; $P < 0.001$), albumin (<3.9 g/dl; $P < 0.001$), GGT (≥50 IU/L; $P < 0.001$), total bilirubin (≥1.0 mg/dl; $P < 0.001$), platelet count ($<20.0 \times 10^4/mm^3$; $P < 0.001$), HBV genotype (C; $P < 0.001$), HBsAg levels (<7,000 IU/ml; $P < 0.001$), HBeAg (positive; $P < 0.001$), HBV DNA (≥5.0 log copies/ml; $P < 0.001$), and HBcrAg (≥3.0 log U/ml; $P < 0.001$). Multivariate analysis that included the above variables identified seven factors that influenced independently the elevated AFP level at the initial visit. These included HBsAg level (<7,000 IU/ml; OR 3.69, $P < 0.001$), albumin (<3.9 g/dl; OR 3.09, $P < 0.001$), platelet count ($<20.0 \times 10^4/mm^3$; OR 2.50, $P = 0.001$), GGT (≥50 IU/L; OR 2.28, $P = 0.001$), AST (≥34 IU/L; OR 2.77, $P = 0.003$), HBeAg (positive; OR 2.07, $P = 0.005$), and HBcrAg (≥3.0 log U/ml; OR 5.10, $P = 0.031$) (Table III).

Among 333 patients with the HBsAg levels less than 500 IU/ml, a high AFP at the initial visit was detected in 42 (12.6%) patients. Univariate analysis identified nine parameters that correlated significantly with a high AFP level at the initial visit. These included AST (≥34 IU/L; $P < 0.001$), ALT (≥43 IU/L; $P = 0.001$), albumin (<3.9 g/dl; $P < 0.001$), GGT (≥50 IU/L; $P < 0.001$), platelet count ($<20.0 \times 10^4/mm^3$; $P = 0.001$), HBV genotype (C; $P < 0.001$), HBeAg (positive; $P < 0.001$), HBV DNA (≥5.0 log copies/ml; $P = 0.001$), and HBcrAg (≥3.0 log U/ml; $P < 0.001$). Multivariate analysis that included the above variables identified three factors that influenced independently the elevated AFP level at the initial visit. These included albumin (<3.9 g/dl; OR 12.8, $P < 0.001$), GGT (≥50 IU/L; OR 6.95, $P = 0.002$), and HBcrAg (≥3.0 log U/ml; OR 5.62, $P = 0.010$) (Table III).

Factors Associated With High AFP Levels at the Initial Visit According to the HBsAg Levels in Patients With Low Transaminase Levels

To minimize the effect of inflammatory activity, we examined the data of 618 (among 1,610 patients) who

TABLE II. Profiles and Laboratory Data of Patients Infected With HBV According to the HBsAg Level at the Initial Visit

	HBsAg <500 IU/L	HBsAg ≥500 IU/L	*P*
Demographic data			
Number of patients	333	1,277	
Sex (male/female)	227/106	820/457	NS
Age (years)*	49 (18–75)	38 (18–83)	<0.001
Family history of liver disease[a]	130 (39.0%)	706 (55.3%)	<0.001
Lifetime cumulative alcohol intake (≥500 kg)	32 (9.6%)	80 (6.3%)	0.037
Laboratory data*			
Total bilirubin (mg/dl)	0.7 (0.2–2.9)	0.6 (0.1–2.9)	0.033
Aspartate aminotransferase (IU/L)	29 (12–175)	40 (5–220)	<0.001
Alanine aminotransferase (IU/L)	32 (7–289)	56 (5–297)	<0.001
Albumin (g/dl)	4.2 (1.1–5.6)	4.2 (1.0–5.5)	NS
Gamma-glutamyl transpeptidase (IU/L)	36 (2–2,370)	38 (4–1,638)	NS
Hemoglobin (g/dl)	14.4 (8.4–17.4)	14.6 (6.9–18.2)	NS
Platelet count ($\times 10^4/mm^3$)	19.1 (2.7–39.6)	19.2 (3.1–44.7)	NS
Alpha-fetoprotein (μg/L)	4 (1–968)	4 (1–1,770)	0.005
Virological data			
HBeAg (No. of positive)	37 (11.1%)	653 (51.1%)	<0.001
HBsAg (IU/ml)*	123 (0.09–498)	4,680 (503 to >125,000)	<0.001
HBcrAg (log U/ml)*	<3.0 (<3.0 to >6.8)	5.9 (<3.0 to >6.8)	<0.001
HBV DNA (log copies/ml)*	3.7 (<2.1 to >9.1)	6.6 (<2.1 to >9.1)	<0.001
HBV genotype (A/B/C/others/ND)	7/104/141/0/81	58/114/978/6/121	<0.001

NS; not significant.
Data are number/percentages of patients, except those denoted by *, which represent the median (range) values.
[a]Family history of positivity for hepatitis B surface antigen including third-degree relatives.