Kawaoka T, Suzuki F, Akuta N, Suzuki Y, Arase Y, Sezaki H, Kawamura Y, Hosaka T, Kobayashi M, Ikeda K, Kumada H. 2007. Efficacy of lamivudine therapy in elderly patients with chronic hepatitis B infection. J Gastroenterol 42:395–401.

Kobayashi S, Shimada K, Suzuki H, Tanikawa K, Sata M. 2000. Development of a new method for detecting a mutation in the gene encoding hepatitis B virus reverse transcriptase active site (YMDD motif). Hepatol Res 17:31–42.

Kobayashi M, Suzuki F, Akuta N, Hosaka T, Sezaki T, Yatsuju H, Yatsuji H, Kobayashi M, Suzuki Y, Arase Y, Ikeda K, Watahiki S, Iwasaki S, Miyakawa Y, Kumada H. 2007. Loss of hepatitis B surface antigen from the serum of patients with chronic hepatitis treated with lamivudine. J Med Virol 79:1472–1477.

Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Wu PC, Dent JC, Barber J, Stephenson SL, Gray DF, for the Asia Hepatitis Lamivudine Study Group. 1998. A one year trial of lamivudine for chronic hepatitis B. N Engl J Med 339:61–68.

Lee WM. 1997. Hepatitis B virus infection. N Engl J Med 337:1733–1745.

Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM. 2000. Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. Hepatology 30:567–572.

Liaw YF, Sung JJY, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J, for the Cirrhosis Asian Lamivudine Multicentre Study Group. 2004. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 351:1521–1531.

Marcellin P, Chang TT, Lim SGL, Sievert W, Tong M, Arterburn S, Borroto-Esoda K, Frederick D, Rousseau F. 2008. Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. Hepatology 48:750–758.

Marcus EL, Tur-Kaspa R. 1997. Viral hepatitis in older adults. J Am Geriatr Soc 45:755–763.

Nafa S, Ahmed S, Tavan D, Pichoud C, Berby F, Stuyver L, Johnson M, Merle P, Abidi H, Trepo C, Zoulim F. 2003. Early detection of viral resistance by determination of hepatitis B virus polymerase mutations in patients treated by lamivudine for chronic hepatitis B. Hepatology 32:1078–1088.

Ooga H, Suzuki F, Tsubota A, Arase Y, Suzuki Y, Akuta N, Sezaki H, Hosaka T, Someya T, Kobayashi M, Saitoh S, Ikeda K, Kobayashi M, Matsuda M, Satoh J, Kumada H. 2004. Efficacy of lamivudine treatment in Japanese patients with hepatitis B virus-related cirrhosis. J Gastroenterol 39:1078–1084.

Patton HM, Lavine JE, Natta MLV, Schwimmer JB, Kleiner D. 2008. Clinical correlates of histopathology in pediatric nonalcoholic steatohepatitis. Gastroenterology 135:1961–1971.

Song B-C, Suh DJ, Lee HC, Chung Y-H, Lee YS. 2000. Hepatitis B e antigen seroconversion after lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. Hepatology 32:803–806.

Suzuki F, Tsubota A, Arase Y, Suzuki Y, Akuta N, Hosaka T, Someya T, Kobayashi M, Saitoh S, Ikeda K, Matsuda M, Satoh J, Takagi K, Kumada H. 2003. Efficacy of lamivudine therapy and factors associated with emergence of resistance in chronic hepatitis B virus infection in Japan. Intervirology 46:182–189.

Suzuki F, Akuta N, Suzuki Y, Sezaki H, Arase Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Ikeda K, Kobayashi M, Matsuda M, Satoh J, Watahiki S, Kumada H. 2006. Clinical and virological features of non-breakthrough and severe exacerbation due to lamivudine-resistant hepatitis B virus mutants. J Med Virol 78:341–352.

Suzuki F, Akuta N, Suzuki Y, Sezaki H, Sezaki H, Arase Y, Kawamura Y, Hosaka T, Kobayashi M, Ikeda K, Kobayashi M, Watahiki S, Kumada H. 2007. Selection of a virus strain resistant to entecavir in a nucleoside-naïve patient with hepatitis B of genotype H. J Clin Virol 39:149–152.

Tsubota A, Arase Y, Suzuki F, Kobayashi M, Matsuda M, Sato J, Suzuki Y, Akuta N, Sezaki H, Hosaka T, Someya T, Kobayashi M, Saitoh S, Ikeda K, Kumada H. 2004. Severe acute exacerbation of liver disease may reduce or delay emergence of YMDD motif mutants in long-term lamivudine therapy for hepatitis B e antigen-positive chronic hepatitis B. J Med Virol 73:7–12.

Yu MW, Shih WL, Lin CL, Liu CJ, Jian JW, Tsai KS, Chen CJ. 2008. Body-mass index and progression of hepatitis B: A population-based cohort study in men. J Clin Oncol 26:5576–5582.

Yuan JM, Govindarajan S, Arakawa K, Yu MC. 2004. Synergism of alcohol, diabetes, and viral hepatitis on the risk of hepatocellular carcinoma in blacks and whites in the U.S. Cancer 101:1009–1017.

Yuen MF, Sablon E, Hui CK, Yuan HJ, Decraemer H, Lai CL. 2001. Factors associated with hepatitis B virus DNA breakthrough in patients receiving prolonged lamivudine therapy. Hepatology 34:785–791.

Zoulim F, Perrillo R. 2008. Hepatitis B: Reflections on the current approach to antiviral therapy. J Hepatol 48:S2–S19.

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Case Report

De novo hepatitis B virus infection in hepatocellular carcinoma following eradication of hepatitis C virus by interferon therapy

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Epidemiological studies have revealed that hepatocellular carcinoma (HCC) is still observed in hepatitis C virus (HCV)-positive patients with a sustained response to interferon (IFN) treatment, although a substantial decrease in the incidence of hepatocellular carcinoma (HCC) has been achieved in those patients. Why HCC develops in patients who have a complete clearance of HCV remains unclear. Here, we provided evidence of latent hepatitis B virus (HBV) infection in an initially HCV-positive chronic hepatitis patient who developed HCC after the complete eradication of HCV by IFN therapy. Although he was initially negative for anti-hepatitis B surface antigen (HBsAg) or circulating HBV DNA but positive for anti-hepatitis B core antigen (anti-HBc) in his sera, he developed HBsAg and HBV DNA during the course of the management of a series of cancers. HBV DNA was detectable in the liver tissues before HBV reactivation and the viral sequences derived from his anti-HBc-positive liver showed 100% homology to that from the serum after HBsAg appearance. These findings indicates that HCV-positive individuals who are positive for anti-HBc in the absence of HBsAg could have latent HBV infection in their liver tissues and intrahepatic HBV infection may play a pivotal role in the development of HCC after the IFN-mediated eradication of HCV.

Key words: hepatitis B virus, hepatitis C virus, hepatocellular carcinoma, interferon

INTRODUCTION

DE NOVO HEPATITIS B virus (HBV) infection is defined as the latent viral infection in individuals that lack any of the serological markers for HBV antigens such as anti-hepatitis B surface antigen (HBsAg).[1] In most cases, however, anti-hepatitis B core antigen (anti-HBc) is frequently detectable and, thus, positivity for anti-HBc is believed to be a surrogate marker for *de novo* HBV infection. Although the clinical significance of *de novo* HBV infection has long been an object of study, direct evidence of actual HBV infection in the liver tissue of patients with positive for anti-HBc but negative for HBsAg is lacking.

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The transmission of HBV to transplant recipients via liver grafts gave the first evidence of the latent HBV infection in HBsAg-negative but anti-HBc-positive individuals.[2] We have demonstrated that the majority of healthy individuals positive for anti-HBc were latently infected by the episomal form of HBV with ongoing viral replication, and could be the high risk source of HBV transmission to the living donor-related liver transplant recipients.[3] In agreement with our report, several studies revealed the HBV transmission from anti-HBc-positive donors via liver transplant.[4,5] In contrast to the *de novo* HBV infection after liver transplantation, the actual prevalence of latent HBV infection in anti-HBc-positive patients with hepatitis C virus (HCV)-related chronic liver disease remains controversial. However, numbers of studies have reported that HBV DNA is frequently detected in liver tumors in anti-HBc-positive, HBsAg-negative patients with HCV-related chronic liver disease.[6–9] In addition, anti-HBc is detectable in approximately 50% of patients with HCV-related chronic liver disease and the proportion of anti-HBc-positive patients

increased in association with the progression of liver disease.[10] Notably, our recent prospective observation study on 872 patients with chronic HCV infection demonstrated that anti-HBc-positive results on serological testing are a marker of high risk for hepatocellular carcinoma (HCC) in patients with HCV-related cirrhosis.[11] These findings suggested that prevalence of occult HBV infection is substantially high among patients with HCV-related chronic liver disease and the latently infected HBV might be involved in the development of HCC. *De novo* HBV infection is therefore of particular concern in this subset of patients, since it might provide the clue of the pathogenesis of HCV-related HCC. To support the possibility that latently infected HBV is indeed present in the liver of patients with chronic HCV infection and could play a role on hepatocarcinogenesis, we reported here the reactivation of latently infected HBV in an initially HCV-positive chronic hepatitis patient who developed HCC after eradication of HCV by interferon (IFN) therapy.

CASE REPORT

A 69-YEAR-OLD MAN attended our hospital in September 1992 for chronic liver dysfunction that had been detected during a regular checkup at his workplace. He showed an elevated level of alanine aminotransferase (107 U/L), was positive for HCV infection, and was diagnosed with chronic HCV (Table S1). He lacked any risk factors for HCC development, as evidenced by the absence of alcoholism and smoking, history of diabetes mellitus, or obesity. In February 1993, he began receiving IFN therapy (subcutaneous 6×10^6 units of IFN-α-2b, three times per week) every 24 weeks. In February 1994, we confirmed the sustained disappearance of serum HCV RNA by qualitative polymerase chain reaction (PCR) assay after the cessation of antiviral therapy. His blood tests showed normal liver function.

Five years after the clearance of HCV infection, abdominal ultrasonography and enhanced-computed tomography detected two hepatic tumors, 3.6 cm and 2.0 cm in diameter. Although his serum α-fetoprotein concentration was within the normal limit at that time, we suspected that these lesions were HCC and a right lobectomy was performed in October 1998.

Histological observation revealed that both tumors were moderately differentiated HCC with a trabecular pattern at stage II (T2N0M0), while the surrounding nontumorous liver tissues showed no signs of liver inflammation or other abnormalities. The next year, he was diagnosed with advanced colorectal cancer in the sigmoid colon. He received a sigmoidectomy and histological findings revealed that the tumor was moderately differentiated colorectal cancers at stage IIa (T3N0M0).

At the time of HCC diagnosis he was initially positive for anti-HBc and anti-HBs, but negative for HBsAg and HBV DNA in his serum. Immunohistochemical evaluation showed no hepatitis viral activities in the liver samples. During the management of a series of cancers, his serological level of HBsAg began to increase spontaneously and the serum HBV DNA and HBsAg became positive in June 2004. Although HBV DNA was not detectable initially in his serum by nested-PCR, it was detectable in the tumor and the surrounding nontumorous tissues of the liver specimens, suggesting that he had been latently infected with HBV in the liver tissues at the time of the operation for HCC. Before the appearance of HBsAg in his serum, he had never received blood product transfusion and he lacked any other risk factors for viral transmission such as intravenous drug injection or high risk of sexual transmission (Figure 1).

To confirm that the HBV appearing in his serum was derived from the viruses initially infecting his liver tissues, we employed sequencing analysis to compare the nucleotide sequences of viral strains in the serum

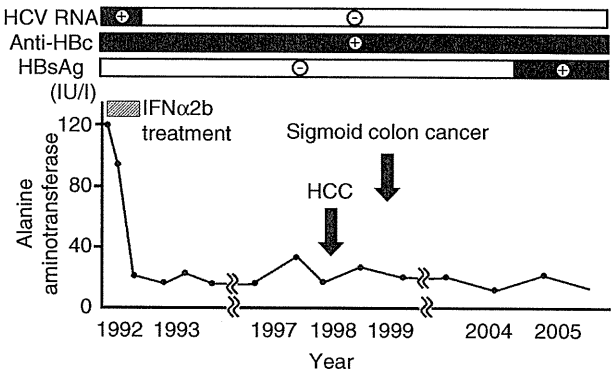

Figure 1 Clinical course of *de novo* hepatitis B virus (HBV) reactivation in the hepatocellular carcinoma (HCC) patient who was initially given a diagnosis of chronic hepatitis C virus (HCV). The patient initially had HCV-related chronic hepatitis with serological markers positive for anti-hepatitis B core antigen but negative for hepatitis B surface antigen (HBsAg), and developed HCC after the complete eradication of HCV infection by interferon therapy. Although he initially lacked the evidence of circulating HBV in his sera, he developed the appearance of HBsAg and HBV DNA during the course of the management of cancers.

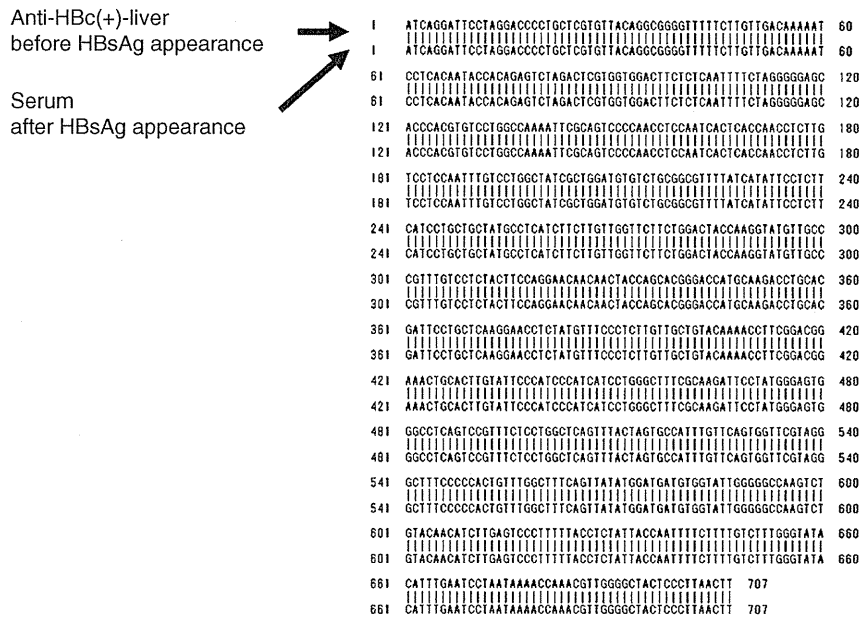

Figure 2 Sequence analyses of hepatitis B virus (HBV) derived from the liver tissue and serum of the hepatocellular carcinoma (HCC) patient who was initially given a diagnosis of chronic hepatitis C virus. The sequence analyses revealed that the nucleotide sequences of HBV surface region derived from the liver tissue at the time of operation for HCC showed 100% homology to that from the serum at the time of hepatitis B surface antigen appearance.

after HBV exacerbation with those detected in the tumor and the surrounding liver tissues at the time of HCC resection. No amplification was obtained by nested PCR using primer set for detection of surface region in the serum sample at the time of operation, HBV DNA was amplified from DNA samples extracted from both the liver tissues obtained at the time of HCC resection and the serum at the time of HBsAg appearance. The detection limit of nested-PCR used in this study was determined as 1×10^2 copies/mL of the circulating viruses.[3] The sequence analyses revealed that the nucleotide sequences of viral surface region derived from the serum showed 100% homology to that from the liver tissues of both cancerous and noncancerous regions (Figure 2), indicating that he had been infected latently with HBV in his liver when he developed HCC, and that HBV reactivation occurred through the clinical course of management of the malignancies.

DISCUSSION

HCC IS A major public health problem worldwide. More than 75% of HCCs are caused by HCV-related chronic liver disease, and nearly 15% are related to HBV-related liver disease.[1,2] Many reports have suggested the possibility that the *de novo* HBV infection may contribute to the development of HCC in HCV-positive patients.[6–8,13,14] However, the role of occult HBV infection in HCV-related HCC is controversial because of the lack of direct evidence showing infection with HBV in the liver of HCV-positive patients. We provided here for the first time the direct evidence of *de novo* HBV infection in the liver tissue of the HCC patient who was initially given a diagnosis of HCV-related chronic liver disease. The patient described here had an HCV-related chronic liver disease with the serological markers positive for anti-HBc but negative for HBsAg, and developed HCC after the complete eradication of HCV infection by IFN therapy. Although he initially lacked any of the serological markers for HBV antigen or circulating HBV DNA in his sera, he developed the appearance of HBsAg and HBV DNA during the course of the management of a series of cancers.

Except the setting of liver transplantation, *de novo* HBV reactivation has been reported in patients after hematopoietic stem cell transplantation and cytotoxic chemotherapy treatment. We previously reported two cases with fatal fuluminant hepatitis caused by *de novo* HBV reactivation.[15,16] Both cases had initially had anti-HBc but not HBsAg and immunosuppressive condition introduced before bone marrow transplantation or chemotherapy for chronic B-cell leukemia resurlted in the reactivation of *de novo* HBV. Recently, Hui *et al.* showed that intensive chemotherapy triggered the development of *de novo* HBV-related hepatitis in eight of 244 lymphoma patients.[17] These findings indicate that the immunosuppressive state caused by malignancies and chemotherapy may result in enhanced repli-

cation of latently infected HBV in the liver tissues, leading to the appearance of HBsAg and HBV DNA in the sera. Although these clinical findings strongly supported the *de novo* HBV reactivation in anti-HBc-positive individuals lacking initial HBsAg, it remained an unsettled question whether the viruses appeared in the sera of patients under immunosuppressive setting was actually derived from their own liver tissues, not from the exogenous origins such as blood transfusion or drug injection during hospitalization. In the current report, we clearly demonstrated that the sequences of the circulating HBV genome after viral reactivation was completely identical to those of the latently infected HBV DNA detected in the liver tissues of the anti-HBc patient, indicating that latent HBV infection was present in anti-HBc-positive patient with HCV-related chronic liver disease and gave rise the *de novo* HBV reactivation during the clinical course of multiple cancer development. The reason why latently infected HBV was spontaneously reactivated in the current case remains unclear at present, since he never received any intensive chemotherapy against cancers. One possibility is, however, to assume that a series of cancer development might cause the change of immunological status, leading to the viral reactivation. Indeed, it is firmLy established that the immunological capacity of cancer patients can be depressed by the disease itself, and thus cancer patients have impaired immune response and susceptible to any viral infection and/or reactivation.[18]

In conclusion, our case indicates that HCV-positive individuals who are positive for anti-HBc in the absence of HBsAg could have latent HBV infection in their liver tissues. One thing to be noted is that this patient developed HCC after the complete clearance of initially infected HCV by IFN treatment. Interestingly, our recent etiological study revealed that none of the anti-HBc-negative patients with HCV-related chronic hepatitis who had a virological response to IFN therapy developed HCC, whereas HCC was diagnosed in some of the anti-HBc-positive patients with a virological response to anti-HCV therapy.[11] Thus, it is tempting to assume that intrahepatic HBV infection played a pivotal role in the development of HCC after the eradication of HCV in our patient. Indeed, a great deal of solid evidence indicates that occult HBV infection is a risk factor for HCC development.[19–21] Further investigation is needed to determine the mechanisms how occult HBV infection could contribute to hepatocarcinogenesis in patients with positive for anti-HBc.

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REFERENCES

1 Raimondo G, Pollicino T, Cacciola I, Squadrito G. Occult hepatitis B virus infection. *J Hepatol* 2007; **46**: 160–70.
2 Uemoto S, Sugiyama K, Marusawa H *et al.* Transmission of hepatitis B virus from hepatitis B core antibody-positive donors in living related liver transplants. *Transplantation* 1998; **65**: 494–9.
3 Marusawa H, Uemoto S, Hijikata M *et al.* Latent hepatitis B virus infection in healthy individuals with antibodies to hepatitis B core antigen. *Hepatology* 2000; **31**: 488–95.
4 Prieto M, Gomez MD, Berenguer M *et al.* De novo hepatitis B after liver transplantation from hepatitis B core antibody-positive donors in an area with high prevalence of anti-HBc positivity in the donor population. *Liver Transpl* 2001; **7**: 51–8.
5 Manzarbeitia C, Reich DJ, Ortiz JA, Rothstein KD, Araya VR, Munoz SJ. Safe use of livers from donors with positive hepatitis B core antibody. *Liver Transpl* 2002; **8**: 556–61.
6 Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. *N Engl J Med* 1999; **341**: 22–6.
7 Shintani Y, Yotsuyanagi H, Moriya K *et al.* The significance of hepatitis B virus DNA detected in hepatocellular carcinoma of patients with hepatitis C. *Cancer* 2000; **88**: 2478–86.
8 Squadrito G, Pollicino T, Cacciola I *et al.* Occult hepatitis B virus infection is associated with the development of hepatocellular carcinoma in chronic hepatitis C patients. *Cancer* 2006; **106**: 1326–30.
9 Tamori A, Nishiguchi S, Shiomi S *et al.* Hepatitis B virus DNA integration in hepatocellular carcinoma after interferon-induced disappearance of hepatitis C virus. *Am J Gastroenterol* 2005; **100**: 1748–53.
10 Marusawa H, Osaki Y, Kimura T *et al.* High prevalence of anti-hepatitis B virus serological markers in patients with hepatitis C virus related chronic liver disease in Japan. *Gut* 1999; **45**: 284–8.
11 Ikeda K, Marusawa H, Osaki Y *et al.* Antibody to hepatitis B core antigen and risk for hepatitis C-related hepatocellular carcinoma: a prospective study. *Ann Intern Med* 2007; **146**: 649–56.
12 Kiyosawa K, Umemura T, Ichijo T *et al.* Hepatocellular carcinoma: recent trends in Japan. *Gastroenterology* 2004; **127**: S17–26.
13 Paterlini P, Brechot C. The detection of hepatitis B virus (HBV) in HBsAG negative individuals with primary liver cancer. *Dig Dis Sci* 1991; **36**: 1122–9.

14 Sheu JC, Huang GT, Shih LN *et al.* Hepatitis C and B viruses in hepatitis B surface antigen-negative hepatocellular carcinoma. *Gastroenterology* 1992; **103**: 1322–7.
15 Iwai K, Tashima M, Itoh M *et al.* Fulminant hepatitis B following bone marrow transplantation in an HBsAg-negative, HBsAb-positive recipient; reactivation of dormant virus during the immunosuppressive period. *Bone Marrow Transplant* 2000; **25**: 105–8.
16 Marusawa H, Imoto S, Ueda Y, Chiba T. Reactivation of latently infected hepatitis B virus in a leukemia patient with antibodies to hepatitis B core antigen. *J Gastroenterol* 2001; **36**: 633–6.
17 Hui CK, Cheung WW, Zhang HY *et al.* Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. *Gastroenterology* 2006; **131**: 59–68.
18 Mera JR, Whimbey E, Elting L *et al.* Cytomegalovirus pneumonia in adult nontransplantation patients with cancer: review of 20 cases occurring from 1964 through 1990. *Clin Infect Dis* 1996; **22**: 1046–50.
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* 2001; **34**: 194–203.
20 Torbenson M, Thomas DL. Occult hepatitis B. *Lancet Infect Dis* 2002; **2**: 479–86.
21 Donato F, Gelatti U, Limina RM, Fattovich G. Southern Europe as an example of interaction between various environmental factors: a systematic review of the epidemiologic evidence. *Oncogene* 2006; **25**: 3756–70.

SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found in the online version of this article:

Table S1 Laboratory data before receiving the interferon therapy

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Novel classification of acute liver failure through clustering using a self-organizing map: usefulness for prediction of the outcome

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Abstract
Background Patients with acute liver failure are classified according to the interval between the onset of hepatitis symptoms and the development of hepatic encephalopathy. We examined the validity of such classifications.
Methods The subjects were 1,022 patients enrolled in a nationwide survey in Japan. The intervals between the onset of the hepatitis symptoms and the development of encephalopathy were 10 days or less in 472 patients (group-A), between 11 and 56 days in 468 patients (group-B), and longer than 56 days in 82 patients (group-C). Data on a total of 104 items collected from the patients were subjected to clustering using a self-organizing map.
Results The patients were classified into three clusters. The first cluster consisted of 411 patients (group-A: 57%, group-B: 39%, group-C: 4%). Their incidence of complications was low; 34% underwent liver transplantation (LT), and their survival rate was 90%, while 94% of those treated without transplant were rescued. The second cluster consisted of 320 patients (21, 65, and 14% groups A, B, and C, respectively), who showed a high incidence of complications; the survival rate was 7% in the patients treated conservatively without LT. Sixteen percent underwent LT and survival rate of these patients was 52%. There was a third cluster, of 291 patients (59, 34, and 7% groups A, B, and C, respectively). Without LT, 81% of the patients died. Seven percent were treated by LT and their survival rate was 60%.
Conclusions Clustering revealed that patients with acute liver failure could be classified into three clusters independent of the interval between the onset of disease symptoms and the development of encephalopathy. This technique may be useful, since the outcomes of the patients differed markedly among the clusters.

Keywords Hepatic encephalopathy · Fulminant hepatitis · Data-mining · Artificial neural network · Liver transplantation

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Abbreviations

LOHF	Late-onset hepatic failure
LT	Liver transplantation
DIC	Disseminated intravascular coagulation
SOM	Self-organizing map
HBV	Hepatitis B virus
HAV	Hepatitis A virus

Introduction

In Japan, fulminant hepatitis is diagnosed when grade II or more severe hepatic encephalopathy develops with a prothrombin time of <40% of the standardized value. In Japan,

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as well as in the United States and Europe, patients with acute liver failure are classified according to the interval between the onset of hepatitis symptoms and the development of hepatic encephalopathy [1–5]. In Japan, fulminant hepatitis is classified into two types; an acute type, in which the encephalopathy develops within 10 days after the onset of the symptoms of hepatitis, and a subacute type, in which the encephalopathy develops between 11 days and 8 weeks after the onset of the hepatitis symptoms [1–3]. Also, patients showing a prothrombin time of <40% of the standardized values in whom hepatic encephalopathy develops between 8 and 24 weeks of the onset of the hepatitis symptoms are diagnosed as having late-onset hepatic failure (LOHF) [1, 3]. Thus, fulminant hepatitis and LOHF, respectively, are almost synonymous with the usage of the terms acute liver failure and LOHF, respectively, in the United States and Europe [4, 5], except that patients without histological evidence of hepatitis, such as those with toxic and ischemic liver injuries, are excluded from both disease conditions.

Although the definition and classifications of acute liver failure in Japan differ from those in Europe and the United States, the Japanese classifications are considered to be useful for prediction of the prognosis of the patients [1–3]. Thus, the indications for liver transplantation (LT) in patients with acute liver failure are currently determined according to the guideline published by the Acute Liver Failure Study Group of Japan in 1996, which includes the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy as one of the parameters in the criteria [6]. Recently, however, a decrease in the predictive accuracy of the guideline was shown when it was adopted for patients seen later than 1998 [7]. Also, in the American Association for the Study of Liver Diseases (AASLD) position paper [4], regarding the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy, the following statement was made: "terms used signifying length of illness such as hyperacute (<7 days), acute (7–21 days) and subacute (>21 days and <26 weeks) are not particularly helpful since they do not have prognostic significance distinct from the cause of the illness".

Several factors other than the disease type, depending on the interval from the onset of the hepatitis symptoms to the development of encephalopathy, may influence the outcome of patients with hepatitis, including the age of the patient, the etiology of hepatitis, liver function tests such as the prothrombin time and serum bilirubin concentration at the onset of hepatic encephalopathy, and the presence of complications such as bacterial infections, renal and cardiac failure, gastrointestinal bleeding, and disseminated intravascular coagulation (DIC) [3, 6]. These data prompted us to reevaluate, by clustering analysis, the validity of the disease type for determination of the outcome in patients with acute liver failure. We applied a self-organizing map (SOM), one of the data-mining methods introduced by Kohonen [8] as an artificial neural network, which has been shown to be suitable for analyses of complex multidimensional relationships in various medical science fields [9–15].

Patients and methods

Patients

The subjects of this study were 1,022 patients with acute liver failure who were enrolled in the nationwide survey performed by the Intractable Liver Diseases Study Group of Japan between 1999 and 2008. All of the patients showed grade II or more severe hepatic encephalopathy and a prothrombin time of <40% of the standardized value and were admitted to 610 hospitals of Japan specializing in hepatology between 1998 and 2007. The interval between the onset of the hepatitis symptoms and the development of encephalopathy was 10 days or less in 472 patients (group-A), between 11 and 56 days in 468 patients (group-B), and more than 56 days in 82 patients (group-C). Group-A, group-B, and group-C patients were diagnosed as having acute-type fulminant hepatitis, subacute-type fulminant hepatitis, and LOHF, respectively, based on the classification used in Japan (Tables 1, 2). All patients were followed up until they either died in hospital or were discharged. They were classified as having survived if they left hospital alive. The patients with hepatitis B virus (HBV) infection were classified into three subgroups according to the serum markers of HBV: (1) transient HBV infection; hepatitis B surface antigen (HBsAg)-positive before the onset of acute liver failure (ALF) or IgM-hepatitis B core antibody (HBcAb)-positive and low titer of IgG-HBcAb, (2) acute exacerbation in HBV carriers; HBsAg-negative before the onset of ALF or IgM-HBcAb-negative or high titer of IgG-HBcAb, and (3) undetermined [neither (1) nor (2)].

Self-organizing map for classification of patients with acute liver failure

Data on a total of 104 items, including the demographic and clinical features, laboratory and imaging data, and the therapies received, were collected from the patients, both before and after the development of grade II or more severe encephalopathy (Table 3). Items such as age, body weight, and biochemical data were analyzed as continuous variables, while those such as gender, outcomes, and complications were analyzed as nominal variables. Clustering analysis was performed through the SOM technique using the IBM Intelligent Miner software (IBM, Tokyo, Japan), as reported in a previous paper [15]. An SOM is a type of

Table 1 Demographic features and outcome and etiology of acute liver failure patients in Japan between 1998 and 2007

	Total (n = 1,022)	Group-A[a] (n = 472)	Group-B (n = 468)	Group-C (n = 82)
Male:female (:unknown)	498:522 (:1)	249:222 (:1)	216:252	33:49
Age (years)	48.3 ± 16.7[b]	46.3 ± 16.3	49.4 ± 17.1[†]	53.8 ± 14.7[†]
HBV carrier [% (n)]	13.5 (127/940)	11.8 (49/417)	16.2 (72/444)*	6.3% (5/79)[#]
Underlying diseases[c] [% (n)]	40.2 (404/1,005)	35.0 (162/463)	43.5 (200/460)*	51.2 (42/82)*,[#]
Previous medication [% (n)]	47.7 (466/977)	41.5 (187/451)	52.5 (234/446)*	56.3 (45/80)*
Etiology [% (n)]				
Viral infection	47.7 (487)	69.3 (327)	31.2 (146)*	17.1 (14)*
HAV	5.4 (55)	10.2 (48)	1.3 (6)*	1.2 (1)*
HBV	39.5 (404)	56.6 (267)	26.9 (126)*	13.4 (11)*
Transient infection	22.8 (233)	40.7 (192)	8.1 (38)*	3.7 (3)*
Carrier	13.1 (134)	10.2 (48)	17.1 (80)*	7.3 (6)[#]
Undetermined	3.6 (37)	5.7 (27)	1.7 (8)*	2.4 (2)
HCV	1.3 (13)	1.3 (6)	1.3 (6)	1.2 (1)
HEV	0.7 (7)	0.4 (2)	1.1 (5)	0 (0)
Other virus	0.8 (8)	0.8 (4)	0.6 (3)	1.2 (1)
Autoimmune hepatitis	7.8 (80)	2.1 (10)	12.0 (56)*	17.1 (14)*
Drug allergy-induced	11.0 (112)	8.1 (38)	13.0 (61)*	15.9 (13)*
Indeterminate	31.2 (319)	18.2 (86)	41.5 (194)*	47.6 (39)*
Insufficient examinations[d]	2.3 (24)	2.3 (11)	2.4 (11)	2.4 (2)

HBV hepatitis B virus, *HAV* hepatitis A virus, *HCV* hepatitis C virus, *HEV* hepatitis E virus

[†] $p < 0.05$ versus group-A by one-way analysis of variance (ANOVA) and multiple comparisons

* $p < 0.05$ versus group-A; [#]$p < 0.05$ versus group-B by χ^2 test and analysis of residuals in cross tabulation

[a] The interval between the onset of the hepatitis symptoms and the development of grade II or more severe hepatic encephalopathy was 10 days or less (group-A), between 11 and 56 days (group-B), and more than 56 days (group-C)

[b] Mean ± SD

[c] Diseases such as metabolic syndrome, malignancy, and psychiatric disorders

[d] The etiology was unknown because of insufficient examinations

Table 2 Outcome of acute liver failure patients in three groups

	% (Number of patients)			
	Total (n = 1,022)	Group-A[a] (n = 472)	Group-B (n = 468)	Group-C (n = 82)
Survival rates in all patients	46.3 (473/1,022)	56.4 (266/472)	39.7* (186/468)	25.6* (21/82)
Treated without liver transplantation	79.4 (811)	86.0 (406)	72.4 (339)	80.5 (66)
Survival rates with medical treatment alone in all patients	30.1 (308/1,022)	46.0 (217/47 2)	17.7* (83/468)	9.8* (8/82)
Survival rates in patients receiving medical therapies alone	38.0 (308/811)	53.4 (217/406)	24.5* (83/339)	12.1* (8/66)
Treated by liver transplantation	20.6 (211)	14.0 (66)	27.6 (129)	19.5 (16)
Survival rates	78.1 (165/211)	74.2 (49/66)	79.7 (103/129)	81.3 (13/16)

* $p < 0.05$ versus cluster 1 and versus cluster 2 by χ^2 test and analysis of residuals in cross tabulation

[a] The interval between the onset of the hepatitis symptoms and the development of grade II or more severe hepatic encephalopathy was 10 days or less (group-A), between 11 and 56 days (group-B), and more than 56 days (group-C)

artificial neural network and topology preserving mappings, consisting of two layers, an input layer and a competitive layer. It provides a low-dimensional representation of multi-dimensional datasets, an array of nodes, called a map. The most important feature of SOM is its preservation of the topological relations among high-dimensional input data, imposed on the output map, usually one or two dimensions, and its technique can be applied to clustering. SOM operates in two phases: training and mapping phases. Training constructs the map using input data, which is a competitive process called vector quantization. A new input vector is classified automatically during the mapping

Table 3 Item characteristics of acute liver failure patients used in the self-organizing map analysis

The types of hepatitis: acute and subacute types of fulminant hepatitis and LOHF
Outcomes: survived and dead among patients treated conservatively without liver transplantation and the patients who underwent transplantation
Gender: male and female
Age (years, continuous variable)
Complications preceding acute liver failure: diseases different from liver diseases such as metabolic syndrome, psychiatric diseases, and malignancies
HBV carrier
Past medical history: operations, blood infusions, alcohol intake, and medications
Family history: liver diseases
Etiology of hepatitis: viral infection, autoimmune hepatitis, drug-induced, indeterminate, and unknown due to insufficient examinations
Serum markers: anti-HAV (IgM), HBs antigen and antibodies, anti-HBc (IgM and IgG), HBV-DNA, anti-HCV, HCV-RNA, anti-HEV (IgM and IgG), HEV-RNA, anti-HDV, HGV-RNA, TTV-DNA, anti-nuclear antibodies, and serum IgG concentration
Interval between the onset of the hepatitis symptoms and the subsequent events (days, continuous variables): development of jaundice and grade II or more severe hepatic encephalopathy, recovery of consciousness, death, and/or transplantation
Interval between the onset of jaundice and the subsequent events (days, continuous variables): development of hepatic encephalopathy of grade II or more, recovery of consciousness, death, and/or liver transplantation
Interval between the onset of grade II or more severe hepatic encephalopathy and the subsequent events (days, continuous variables): recovery of consciousness, death, and/or transplantation
Maximal grade of hepatic encephalopathy (II–V, continuous variable)
Cause of death: liver failure, infection, and other complications
Symptoms at the onset of grade II or more severe hepatic encephalopathy: fever, jaundice, ascites, edema, flapping tremor, halitosis, loss of liver dullness, convulsion, tachycardia, and hyperventilation
Laboratory data at the onset of grade II or more severe hepatic encephalopathy (continuous variables): the grading of the encephalopathy; peripheral counts of WBC and platelets; prothrombin time; hepaplastin test; plasma concentrations of antithrombin III and ammonia; serum concentrations of AST, ALT, total albumin, total bilirubin, direct bilirubin, AFP, and HGF; the serum concentration ratios of direct-to-total bilirubin and the BCAA-to-tyrosine and Fischer ratio
Symptoms and laboratory data 5 days after the onset of encephalopathy (continuous variables): the grading of the encephalopathy, prothrombin time
Atrophy of the liver at the onset of grade II or more severe hepatic encephalopathy, 5 days after the onset of encephalopathy and during the whole course of the disease
Complications of acute liver failure at the onset of grade II or more severe hepatic encephalopathy, 5 days after the onset of encephalopathy and during the whole course of the disease: bacterial and fungal infections, gastrointestinal bleeding, renal failure, cardiac failure, disseminated intravascular coagulation, other complications
Number of complications at the onset of grade II or more severe hepatic encephalopathy, 5 days after the onset of encephalopathy, and during the whole course of the disease (continuous variables)
Hospitals providing medical care and liver transplantation: plasma exchange, hemodiafiltration, glucocorticoids, glucagon and insulin, prostaglandin E1, interferon, lamivudine or entecavir, cyclosporine-A, anticoagulants, fresh frozen plasma, and liver transplantation

LOHF late-onset hepatic failure, *HAV* hepatitis A virus, *HBV* hepatitis B virus, *HDV* hepatitis D virus, *HGV* hepatitis G virus, *TTV* torque teno virus, *WBC* white blood cell count, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *AFP* alpha-fetoprotein, *HGF* hepatocyte growth factor, *BCAA* branched-chain amino acids, *HBs* hepatitis B surface, *HBc* hepatitis B core

phase [16]. The training phase utilizes competitive learning. Unsupervised learning of SOM processes was carried out as follows. First, an input vector was selected randomly from among the 104 items. $\boldsymbol{D}$ was defined as the Euclidean distance between the input vector $\boldsymbol{X}$ and the reference vector $\boldsymbol{W}$. An individual $\boldsymbol{D}$ value was applied to every neuron j.

$$\boldsymbol{D}_j = \sqrt{\sum_{i=1}^{N} \left(\boldsymbol{X}_i - \boldsymbol{W}_{ij}\right)^2} \quad \text{for} \quad j \in L$$

where i is the individual item, N the number of items, j the individual neuron, and L is the number of neurons.

During the mapping phase, a neuron with the minimum $\boldsymbol{D}$ value was determined as the winning neuron c. Reference vectors of the winning and neighbor neurons $N_c(t)$ were modified according to the following formula:

$$\boldsymbol{W}_j(t+1) = \boldsymbol{W}_j(t) + \alpha(t)\left(\boldsymbol{X}(t) - \boldsymbol{W}_j(t)\right) \quad \text{for} \quad j \in N_c(t)$$

where α is the learning rate, initially close to 1 and diminished at each iteration ($0 < \alpha \leq 1$).

SOM processes were terminated when the change in $\boldsymbol{D}$ was less than a certain level. Calculation of cluster aggregation levels on the map resulted in the identification of three major clusters.

Statistical analysis

Statistical testing was performed using SPSS version 15.0J (SPSS, Tokyo, Japan). Results were presented as means ± SD. Continuous variables were compared using one-way factorial analysis of variance (ANOVA) and multiple comparisons with Tukey and Dunnett's T3 methods. Categorical data were compared using the χ^2 test, and further, we carried out an analysis of residuals in cross tabulation.

Results

Demographic and clinical features and outcome of patients with acute liver failure

As shown in Table 1, of the total study population, 40.2% had underlying diseases such as metabolic syndrome, and most of such patients were on daily medications. The etiology of fulminant hepatitis was viral infection in 69.3, 31.2, and 17.1% of the patients in group-A, group-B, and group-C, respectively. In most cases, the causative virus was HBV; transient infection was predominant in group-A, whereas asymptomatic carriers showing acute exacerbation of hepatitis predominated in group-B. The survival rates of the 811 patients who received medical treatment alone (without LT) were 53.4% in group-A, 24.5% in group-B, and 12.1% in group-C (Table 2).

Clustering analysis of the patients with acute liver failure

The results of analysis by the SOM technique revealed that the acute liver failure patients could be classified into three clusters, consisting of 411, 320, and 291 patients, respectively: cluster-1 (40.2%), cluster-2 (31.3%), and cluster-3 (28.5%). The demographic and clinical features of the patients in each cluster are shown in Tables 4, 5, 6, and 7 and Fig. 1.

As shown in Table 4, the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy differed among the three clusters; the percentages of patients belonging to group-A, group-B, and group-C were 56.7, 39.2, and 4.1%, respectively, in cluster-1, and 58.8, 34.0, and 7.2%, respectively, in cluster-3. However, in cluster-2, the percentage of patients belonging to group-A was smaller, and those of patients belonging to group-B and group-C were greater (21.3, 65.0, and 13.8%, respectively) than in either cluster-1 or cluster-3.

Also, the demographic features of the patients differed among the three clusters (Table 5). There were a greater number of females in both cluster-1 and cluster-2 than in cluster-3, while the number of males was higher than that of females in cluster-3. The age (years: mean ± SD) of the patients was significantly higher in cluster-2 (54.9 ± 15.6) than in cluster-1 (41.0 ± 16.2) or cluster-3 (51.5 ± 14.2), and the age in cluster-3 was also significantly higher than that in cluster-1. The age distribution of the patients in each cluster is shown in Fig. 1. The percentage of HBV carriers was especially high in patients in cluster-3 (23.0%) compared with those in cluster-1 (7.9%) and cluster-2 (12.7%). In contrast, the underlying diseases preceding acute liver failure, such as metabolic syndrome, malignancies, and psychiatric disorders, were found more frequently in patients in cluster-2 (54.0%) and cluster-3 (49.3%) than in the patients in cluster-1 (23.3%). Such a tendency was also noted for the percentages of patients receiving previous medications (34.4, 60.1, and 53.7%, respectively, in cluster-1, cluster-2, and cluster-3).

Table 5 shows the relation between the etiology of the liver disease and the classification depending on the SOM analysis in the patients with acute liver failure. Viral infection was the most prevalent etiology in the patients in cluster-1 (53.0%) and cluster-3 (63.6%), but the causative virus differed between these clusters. Although the percentage of patients with transient HBV infection was similar in cluster-1 and cluster-3 (29.4 and 31.6%, respectively), the percentage of HBV carriers with hepatitis exacerbation was higher in cluster-3 (20.6%) as compared

Table 4 Intervals between the onset of hepatitis symptoms and grade II or more severe hepatic encephalopathy in acute liver failure patients and their clusters according to the classification by the self-organizing map

Groups[a]	% (Number of patients)			
	Total (n = 1,022)	Cluster-1 (n = 411)	Cluster-2 (n = 320)	Cluster-3 (n = 291)
Group-A	46.2 (472)	56.7 (233)*	21.3 (68)	58.8 (171)*
Group-B	45.8 (468)	39.2 (161)*	65.0 (208)	34.0 (99)*
Group-C	8.0 (82)	4.1 (17)*,#	13.8 (44)	7.2 (21)*

* $p < 0.05$ versus cluster 2; #$p < 0.05$ versus cluster 3 by χ^2 test and analysis of residuals in cross tabulation

[a] The interval between the onset of the hepatitis symptoms and the development of grade II or more severe hepatic encephalopathy was 10 days or less (group-A), between 11 and 56 days (group-B), and more than 56 days (group C)

Table 5 Demographic features and etiology of acute liver failure patients and their clusters according to the classification by the self-organizing map

	Total (n = 1,022)	Cluster-1 (n = 411)	Cluster-2 (n = 320)	Cluster-3 (n = 291)
Male:female (:unknown)	498:523 (:1)	187:224	122:197 (:1)	189:102*,#
Age (years)	48.3 ± 16.7[a]	41.0 ± 16.2	54.9 ± 15.6†	51.5 ± 14.2†,‡
HBV carrier [% (n)]	13.4 (126/940)	7.9 (31/390)	12.7 (39/306)*	23.0 (56/244)*,#
Underlying diseases[b] [% (n)]	40.2 (404/1,005)	23.3 (95/408)	54.0 (169/313)*	49.3 (140/284)*
Previous medication [% (n)]	47.7 (466/977)	34.4 (139/404)	60.1 (182/303)*	53.7 (145/270)*,#
Etiology [% (n)]				
Viral infection	47.7 (487)	53.0 (218)	26.3 (84)*	63.6 (185)#
HAV	5.4 (55)	9.0 (37)	1.6 (5)*	4.5 (13)*,#
HBV	39.5 (404)	40.1 (165)	22.2 (71)*	57.7 (168)*,#
Transient infection	22.8 (233)	29.4 (121)	6.3 (20)*	31.6 (92)#
Carrier	13.1 (134)	7.5 (31)	13.4 (43)*	20.6 (60)*,#
Undetermined	3.6 (37)	3.2 (13)	2.5 (8)	5.5 (16)*,#
HCV	1.3 (13)	1.7 (7)	1.9 (6)	0.0 (0)*,#
HEV	0.7 (7)	1.2 (5)	0.0 (0)	0.7 (2)
Other virus	0.8 (8)	1.2 (5)	0.3 (1)	0.7 (2)
Autoimmune hepatitis	7.8 (80)	5.4 (22)	15.3 (49)*	3.1 (9)#
Drug allergy-induced	11.0 (112)	9.2 (38)	15.9 (51)*	7.9 (23)*,#
Indeterminate	31.2 (319)	30.2 (124)	40.9 (131)*	22.0 (64)*,#
Insufficient examinations[c]	2.3 (24)	1.9 (8)	1.9 (6)	3.4 (10)

HBV hepatitis B virus, *HAV* hepatitis A virus, *HCV* hepatitis C virus, *HEV* hepatitis E virus

† $p < 0.05$ versus cluster 1; ‡$p < 0.05$ versus cluster 2 by one-way ANOVA and multiple comparisons

* $p < 0.05$ versus cluster 1; #$p < 0.05$ versus cluster 2 by χ^2 test and analysis of residuals in cross tabulation

[a] Mean ± SD

[b] Diseases such as metabolic syndrome, malignancy, and psychiatric disorders

[c] The etiology was unknown because of insufficient examinations

with that in cluster-1 (7.5%). In contrast, the percentages of patients with drug-induced liver injury, autoimmune hepatitis, and those with indeterminate etiology was higher in cluster-2 (15.9, 15.3, and 40.9%, respectively) than in cluster-1 (9.2, 5.4, and 30.2%, respectively) or cluster-3 (7.9, 3.1, and 22.0%, respectively).

As shown in Table 6, the frequencies of complications, such as bacterial infection, gastrointestinal bleeding, renal and cardiac failure, DIC, and cerebral edema, differed among the three clusters. There were no complications in 56.9% of the patients in cluster-1, while the percentages of patients with no complications were as low as 11.9 and 1.4% in cluster-2 and cluster-3, respectively. The percentage of patients with 2 or more complications was 11.9% in cluster-1, which was markedly lower than the values in cluster-2 (54.4%) and cluster-3 (90.4%).

Consequently, the outcome of the patients differed markedly among the three clusters (Table 7). LT was performed in 34.3% of the patients in cluster-1, which was a significantly greater percentage than the corresponding percentages in cluster-2 (15.6%) and cluster-3 (6.9%), and the survival rates of these patients with LT were 90.1, 52.0, and 60.0% in cluster-1, cluster-2, and cluster-3, respectively. Also, the survival rate in the patients not treated by LT was significantly higher in cluster-1 (94.4%) than in cluster-2 (6.7%) or cluster-3 (12.9%). The survival rate among patients receiving medical treatment alone was still significantly higher in cluster-1 (62.0%) than in cluster-2 (5.6%) or cluster-3 (12.0%), even when it was expressed as the ratio of number of survivors to the total number of patients including those receiving liver transplantation.

Discussion

In this study, the validity of the classification of acute liver failure according to the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy was evaluated. Previous classifications were established based on decision analyses with a linear mode, such as multivariate regression analysis. Such analysis, however, is not suitable for modeling complex multidimensional relationships, which may also affect the prognosis of acute liver failure patients in a complex

Table 6 Numbers of complications in acute liver failure patients during the whole course of the disease and their clusters according to the classification by the self-organizing map

Number of complications	% (Number of patients)			
	Total (n = 1,022)	Cluster-1 (n = 411)	Cluster-2 (n = 320)	Cluster-3 (n = 291)
0	27.0 (276)	56.9 (234)	11.9 (38)*	1.4 (4)*
1	25.4 (260)	31.1 (128)	33.8 (108)	8.2 (24)*,#
2	20.3 (207)	10.2 (42)	28.8 (92)*	25.1 (73)*
3	14.2 (145)	1.7 (7)	16.6 (53)*	29.2 (85)*,#
4	7.9 (81)	0.0 (0)	6.6 (21)*	20.6 (60)*,#
5	3.7 (38)	0.0 (0)	2.5 (8)*	10.3 (30)*,#
6	0.9 (9)	0.0 (0)	0.0 (0)	3.1 (9)*,#

* $p < 0.05$ versus cluster 1; #$p < 0.05$ versus cluster 2 by χ^2 test and analysis of residuals in cross tabulation

Table 7 Outcome of acute liver failure patients and their clusters according to the classification by the self-organizing map

	% (Number of patients)			
	Total (n = 1,022)	Cluster-1 (n = 411)	Cluster-2 (n = 320)	Cluster-3 (n = 291)
Survival rates in all patients	46.2 (473/1,022)	92.9 (382/411)	13.8* (44/320)	16.2* (47/291)
Treated without liver transplantation	79.4 (811)	65.7 (270)	84.4 (270)	93.1 (271)
Survival rates with medical treatment alone in all patients	30.1 (308/1,022)	62.0 (255/411)	5.6* (18/320)	12.0* (35/291)
Survival rates in patients receiving medical therapies alone	38.0 (308/881)	94.4 (255/270)	6.7* (18/270)	12.9* (35/271)
Treated by liver transplantation	20.6 (211)	34.3 (141)	15.6 (50)	6.9 (20)
Survival rates	78.1 (165/211)	90.1 (127/141)	52.0* (26/50)	60.0* (12/20)

* $p < 0.05$ versus cluster 1 by χ^2 test and analysis of residuals in cross tabulation

manner. Thus, we evaluated the validity of the classification using artificial neural networks, one of the decision analyses with a non-linear mode.

The SOM is a neural network methodology that has been used for the categorization and interpretation of multidimensional data sets with a large scale [8]. The categorization can be achieved through transformation of an n-dimensional input vector into an r-dimensional separated map, in which r is smaller than n and is usually 2. Thus, the input vectors are preserved on the same area of the map, and these signal processing units are called "neurons". Each neuron shows an association through an n-dimensional reference vector that determines the linkage between the maps of the input and output vectors. The input vector is transformed onto a particular neuron through the calculation in which the n-dimensional distance between the input and reference vectors of the neuron is the minimal distance. Based on such calculations, the reference vectors of the neurons are updated repeatedly, and finally the best-matching neurons are established. As a result, the SOM can accomplish clustering of the input data sets without the experience of specialists. Such a process is called unsupervised learning.

In the present study, the results of the clustering analysis using the SOM revealed that the patients with acute liver failure could be classified into three clusters different from the disease groups, depending on the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy as nominal variables. Also, similar results were obtained in analysis when such intervals were assessed as continuous variables (data not shown). As shown in Table 4, 404 of the 472 patients (85.6%) in group-A belonged to either cluster-1 or cluster-3. Also, while 208 of the 468 patients (44.4%) in group B and 44 of the 82 patients (53.7%) in group-C were classified into cluster-2, a distinct accumulation of the patients in these three clusters, as was the case in group-A, was not found in the other two groups. Moreover, it should be noted that the demographic and clinical features and the outcome differed between the patients in cluster-1 and those in cluster-3 (Tables 5, 6, and 7; Fig. 1), despite the percentages of patients belonging to groups A, B, and C being almost identical in these two clusters (Table 4). These observations strongly suggest that patients with acute liver failure in Japan may be classified into disease types different from those that are exclusively dependent on the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy.

The novel classification through the SOM analysis may be clinically important, since the outcome of the patients

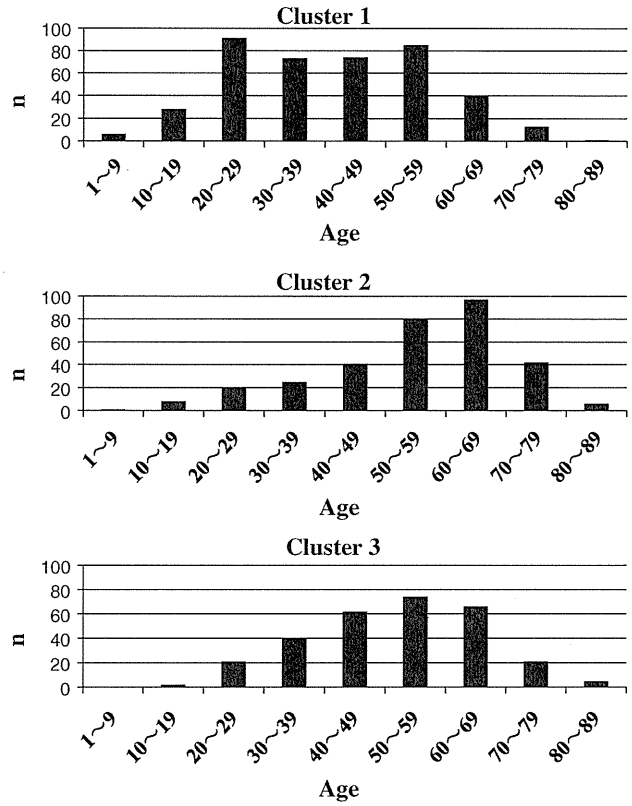

Fig. 1 Distribution of the ages (years) of acute liver failure patients classified according to the clustering analysis based on the self-organizing map

differed more obviously among the clusters as compared with the differences between the disease types determined based only on the interval from the onset of the hepatitis symptoms and the development of hepatic encephalopathy (Table 1). A total of 141 (66.8%) of the 211 patients treated by LT were classified into cluster-1, and the survival rate of these patients was 90.1%. Also, the survival rate of those treated conservatively without LT was extremely high (94.4%) in this cluster. In contrast, the percentage of the patients treated by LT was extremely low in cluster-2 and cluster-3, and 78.8% (252/320) and 81.1% (236/291) of the patients, respectively, who were treated conservatively without LT died. The factors that seemed to be responsible for the differences among these clusters were the sex and age of the patients, the presence of the underlying diseases and history of previous medications, the etiology of the liver diseases, and the number of complications developing during acute liver failure, as well as the interval between the onset of the hepatitis symptoms and the development of hepatic encephalopathy. The patients with such factors, among which complex multi-dimensional relationships may exist, could be classified into the clusters reflecting their outcome only through SOM analysis, a form of artificial neural network.

Our classification is also useful for speculating on the possible cause of acute liver failure in the patients with indeterminate etiology. Of the 319 patients with indeterminate etiology, 131 (41%) were classified into cluster-2, in which 61 and 46% of the patients in whom the acute liver failure was due to autoimmune hepatitis and drug-induced liver injury, respectively, were also classified. The total percentage of patients with indeterminate etiology and those whose acute liver failure was due to autoimmune hepatitis and drug-induced liver injury was 72% in this cluster. In contrast, 124 (39%) of the patients with indeterminate etiology were classified into cluster-1 and 64 (20%) into cluster-3. Viral infection was the most prevalent etiology in both clusters (53 and 64%, respectively, in cluster-1 and cluster-3), but the causative virus differed between these clusters; transient infections of HAV and HBV were dominant in cluster-1, while HBV carriers showing acute hepatitis exacerbation prevailed in cluster-3. Considering the characteristics of the patients with indeterminate etiology in each cluster, possible etiologies in these patients were transient unknown virus infection in cluster-1, autoimmune hepatitis or drug-induced liver injury in cluster-2, and HBV occult infection in cluster-3. These possibilities should be further investigated.

Although our classification may be useful to predict the outcome of patients with acute liver failure and the possible causes of the condition in those with indeterminate etiology, there still exist problems that need to be resolved preceding the application of the system for the benefit of hepatologists in Japan. First, the patients can be classified into the three clusters only through the SOM technique using the IBM Intelligent Miner software. Physicians and/or transplant surgeons are required to input the data sets on a total of 104 items collected from the patients using personal computers, and the clusters that the patients are categorized into are shown on the websites through a blind decision process. Such complicated procedures might prevent our system from becoming prevalent for clinical use by hepatologists nationwide. Simple algorithms, using which any physicians and/or surgeons can predict the outcome of the patients based on the clusters that they belong to, with no reference to the websites, should be established using data-mining analysis methods, such as the decision tree [17]. Secondly, the purpose of our system is to categorize patients with acute liver failure depending on the demographic and clinical features of the patients as well as their prognosis, suggesting that such a system may not always be suitable for determination of the indications for LT. For example, regarding cluster-1, in which the survival rate of the patients was extremely high, one-third of the patients were rescued after being treated by LT, while two-thirds survived following conservative treatment without transplantation. Thus, a system to directly predict

the prognosis of the patients should be established based on the data sets exclusively derived from the patients treated conservatively without LT. The radial basis function model [17, 18] and/or the back propagation model [19], one form of artificial neural network, would seem to be suitable for such analysis with the data-mining procedures. Also, precise information on past medical history, especially regarding underlying diseases and previous medication (which are important factors that determine the character of each cluster), should be analyzed. These projects should be undertaken in the future.

In the present study, patients with acute liver failure diagnosed according to the definition established in Japan were analyzed through the SOM technique. Analyses using the SOM technique in patients with acute liver failure in the United States and Europe also merit consideration, since the outcome prognosis of the patients may be determined through complex multidimensional relationships including the etiology, as well as the intervals between the onset of hepatitis symptoms and the development of hepatic encephalopathy [4, 20, 21]. There seems to be a higher percentage of patients that can be rescued without LT in these countries than in Japan, possibly because of the difference in the definition criteria of acute liver failure, especially in relation to the differences in criteria for prothrombin time among the countries [1–4]. Such patients may be identified through data-mining analysis using the SOM technique. Thus, such an approach may contribute to the overcoming of problems of organ shortage in the field of LT, which also exist in Europe and the United States.

In conclusion, patients with acute liver failure in Japan were classified into three clusters independent of the disease types depending on the interval between the disease onset and the development of encephalopathy, using the SOM technique. This technique may be useful for establishing prognosis prediction models, since the outcome of the patients differed markedly among the clusters.

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References

1. Mochida S, Fujiwara K. Symposium on clinical aspects in hepatitis virus infection. 2. Recent advances in acute and fulminant hepatitis in Japan. Intern Med. 2001;40:175–7.
2. Inuyama Symposium Kiroku Kanko-Kai. Hepatitis type A and fulminant hepatitis. In: The proceedings of the 12th Inuyama symposium. Tokyo: Chugai Igaku-sha; 1982 (in Japanese).
3. Fujiwara K, Mochida S, Matsui A, Nakayama N, Nagoshi S, Toda G, et al. Fulminant hepatitis and late onset hepatic failure in Japan: summary of 698 patients between 1998 and 2003 analyzed in annual nationwide survey. Hepatol Res. 2008;38:646–57.
4. Polson J, Lee WM. AASLD position paper: the management of acute liver failure. Hepatology. 2005;41:1179–97.
5. Gimson AES, O'Grady JG, Ede RJ, Portmann B, Williams R. Late onset hepatic failure: clinical, serological and histological features. Hepatology. 1986;6:288–94.
6. Sugihara J, Naito T, Ishiki Y, Murakami N, Naiki T, Koshino Y, et al. A multicenter study on the prognosis and indication of liver transplantation for fulminant hepatitis in Japan: details of decision of the guideline for liver transplantation in Japanese Acute Hepatic Failure Study Group (1996). Acta Hepatologica Japonica. 2001;42:543–57 (in Japanese).
7. Mochida S, Nakayama N, Matsui A, Nagoshi S, Fujiwara K. Re-evaluation of the Guideline published by the Acute Liver Failure Study Group of Japan in 1996 to determine the indications of liver transplantation in patients with fulminant hepatitis. Hepatol Res. 2008;38:970–9.
8. Kohonen T. Self-organizing maps. Berlin: Springer; 2001.
9. Talbi ML, Charef A. PVC discrimination using the QRS power spectrum and self-organizing maps. Comput Methods Programs Biomed. 2009;94:223–31.
10. Basara HG, Yuan M. Community health assessment using self-organizing maps and geographic information systems. Int J Health Geogr. 2008;7:67.
11. Tsunedomi R, Iizuka N, Hamamoto Y, Uchimura S, Miyamoto T, Tamesa T, et al. Patterns of expression of cytochrome P450 genes in progression of hepatitis C virus-associated hepatocellular carcinoma. Int J Oncol. 2005;27:661–7.
12. Haydon GH, Hiltunen Y, Lucey MR, Collett D, Gunson B, Murphy N, et al. Self-organizing maps can determine outcome and match recipients and donors at orthotopic liver transplantation. Transplantation. 2005;79:213–8.
13. Omori K, Terai S, Ishikawa T, Aoyama K, Sakaida I, Nishina H, et al. Molecular signature associated with plasticity of bone marrow cell under persistent liver damage by self-organizing-map-based gene expression. FEBS Lett. 2004;578:10–20.
14. Gebbinck MS, Verhoeven JT, Thijssen JM, Schouten TE. Application of neural networks for the classification of diffuse liver disease by quantitative echography. Ultrason Imaging. 1993;15:205–17.
15. Takasaki S, Kawamura Y, Konagaya A. Selecting effective siRNA sequences based on the self-organizing map and statistical techniques. Comput Biol Chem. 2006;30:169–78.
16. Wu H, Chen X, Li Z, Wang S, Cui W, Meng Q. Identifying spatial patterns of land use and cover change at different scales based on self-organizing map. In: Zeng Z, Wang J, editors. Advances in neural network research and applications. Lecture notes in Electrical Engineering, vol. 67. Berlin: Springer; 2010. p. 355–61.
17. Takasaki S, Kawamura Y, Konagaya A. Selecting effective siRNA sequences by using radial basis function network and decision tree learning. BMC Bioinformatics. 2006;7(Suppl 5):S22.
18. Poggio T, Girosi F. Networks for approximation and learning. Proc IEEE. 1990;78:1481–97.
19. Werbos PJ. Intelligence in the brain: a theory of how it works and how to build it. Neural Netw. 2009;22:200–12.
20. Dhiman RK, Seth AK, Jain S, Chawla YK, Dilawari JB. Prognostic evaluation of early indicators in fulminant hepatic failure by multivariate analysis. Dig Dis Sci. 1998;43:1311–6.
21. Gotthardt D, Riediger C, Weiss KH, Encke J, Schemmer P, Schmidt J, et al. Fulminant hepatic failure: etiology and indications for liver transplantation. Nephrol Dial Transplant. 2007;22(Suppl 8):viii5–8.

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Special Report

Diagnostic criteria of acute liver failure: A report by the Intractable Hepato-Biliary Diseases Study Group of Japan

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The diagnostic criteria of fulminant hepatitis in Japan are different from those of acute liver failure in Europe and the United States, both in regard to the histological features in the liver and the cutoff values of the prothrombin time. Thus, the Intractable Hepato-Biliary Disease Study Group established novel diagnostic criteria for "acute liver failure" in Japan based on the demographic and clinical features of the patients. Patients showing prothrombin time values of 40% or less of the standardized values or international normalized ratios of 1.5 or more caused by severe liver damage within 8 weeks of onset of the symptoms are diagnosed as having "acute liver failure", where the liver function prior to the current onset of liver damage is estimated to be normal. Acute liver failure is classified into "acute liver failure without hepatic coma" and "acute liver failure with hepatic coma," depending on the severity of the hepatic encephalopathy; the latter is further classified into two types, the "acute type" and the "subacute type", in which grade II or more severe hepatic coma develops within 10 days and between 11 and 56 days, respectively, after the onset of disease symptoms. Patients without histological findings of hepatitis, such as those with liver damage caused by drug toxicity, circulatory disturbance or metabolic disease, are also included in the disease entity of "acute liver failure", while acute-on-chronic liver injuries, such as liver injury caused by alcohol, are excluded. A nationwide survey of "acute liver failure" in Japan based on the novel criteria is proposed.

Key words: acute liver failure, diagnostic criteria, fulminant hepatitis, hepatic encephalopathy, late onset hepatic failure

INTRODUCTION

DIFFERENCES EXIST IN the demographic and clinical features of patients with acute liver failure between Japan and Europe and/or the United States. Hepatitis viral infection is the most important and frequent cause of acute liver failure in Japan,[1] while acetaminophen-induced toxic liver injury prevails as the major cause of acute liver failure in Europe and the United States.[2] Thus, acute liver failure has been typically represented by fulminant hepatitis in Japan, and the diagnostic criteria for "fulminant hepatitis", which were different from those for "acute liver failure" in Europe and the United States, were established by the Inuyama Symposium in 1981.[3] According to the Inuyama Symposium criteria, patients with hepatitis were diagnosed as having fulminant hepatitis when they developed grade II or more severe hepatic encephalopathy within 8 weeks of the onset of hepatitis symptoms due to severe liver damage as represented by prothrombin time values of 40% or less as compared to the standardized values. Fulminant hepatitis was further classified into two disease types, acute and subacute types of fulminant hepatitis, on the basis of the

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Table 1 Diagnostic criteria for fulminant hepatitis in Japan established by the Intractable Liver Diseases Study Group of Japan, the Ministry of Health, Welfare and Labour (2003)

Fulminant hepatitis is defined as hepatitis with hepatic encephalopathy of grade II or more, develops in patients within 8 weeks of the onset of disease symptoms, and is associated with severe derangement of the liver function, including prothrombin time values of less than 40% of the standardized values. Fulminant hepatitis is classified into two subtypes: the acute type and the subacute type, according to whether the encephalopathy occurs within 10 days and later than 11 days, respectively, after the onset of the symptoms.

Note 1: Patients with chronic liver diseases are excluded from the disease entity of fulminant hepatitis, but asymptomatic hepatitis B virus (HBV) carriers developing acute exacerbation are included as cases of fulminant hepatitis.

Note 2: Acute liver failure with no histological evidence of liver inflammation, such as that caused by drug or chemical intoxication, circulatory disturbance, acute fatty liver of pregnancy or Reye's syndrome are excluded from fulminant hepatitis.

Note 3: The grading of hepatic encephalopathy is based on the criteria presented in the Inuyama Symposium in 1972.

Note 4: The etiology of fulminant hepatitis is based on the criteria established by the Intractable Liver Diseases Study Group of Japan in 2002.

Note 5: Patients with no or grade I encephalopathy, but showing prothrombin time values of less than 40% of the standardized values are diagnosed as having acute hepatitis, severe type. Patients in whom the encephalopathy develops between 8 and 24 weeks after the disease onset, with prothrombin time values of less than 40% of the standardized values are diagnosed as having late-onset hepatic failure (LOHF). Both are diseases related to fulminant hepatitis, but are regarded differently from fulminant hepatitis.

hepatic encephalopathy developing within 10 days and between 11 and 56 days, respectively, after the onset of the hepatitis symptoms. Fulminant hepatitis in Japan is defined as acute liver failure with histological evidence of hepatic inflammation, characterized by lymphocytic infiltration of the liver. Thus, the etiology of fulminant hepatitis includes viral infections, hepatitis B virus (HBV) carriers, autoimmune hepatitis, drug allergy-induced liver injuries, and hepatitis of indeterminate etiologies, but would exclude liver injuries caused by drug toxicity, circulatory disturbances, metabolic diseases, acute fatty liver of pregnancy, and postoperative liver damage, all of which are also included as etiological factors for disease entity of "acute liver failure" in Europe and the United States. Also, the extent of liver damage has been defined worldwide based on the degree of prolongation of the prothrombin time although the values are expressed as a percentage of the standardized values in Japan, but as the international normalized ratio (INR) in the United States.[2] Therefore, the diagnostic criteria for "fulminant hepatitis" in Japan need to be revised to correspond to those for "acute liver failure" in Europe and the United States.

The Intractable Liver Diseases Study Group of Japan, supported by the Ministry of Health, Labour and Welfare last revised the diagnostic criteria for "fulminant hepatitis" in 2002 (Table 1).[1,4] The definition and concept of fulminant hepatitis, however, were not modified, except that five items clarifying the inclusion and exclusion criteria for fulminant hepatitis were added to the footnotes. Thus, in 2006, the Intractable Hepato-Biliary Diseases Study Group of Japan (formerly the Intractable Liver Diseases Study Group of Japan) constituted a task force to establish novel diagnostic criteria for "acute liver failure", which includes the disease entity of "fulminant hepatitis". We report on the established criteria for "acute liver failure" available for Japanese patients.

METHODS

Expression of prothrombin time

A QUESTIONNAIRE WAS sent to active members of the Japan Society of Hepatology affiliated to 550 departments of gastroenterology and/or hepatology in 454 institutions in Japan, to determine what type of commercial kit they used for prothrombin time measurement in their clinical laboratories. Then, information regarding the International Sensitivity Index (ISI) values and INR values corresponding to 40% of the standardized values for each kit was collected from the respective industrial companies.

Evaluation of acute liver failure other than fulminant hepatitis in Japan

A questionnaire was sent to specialists of the Japanese Association of Acute Medicine in 533 institutions, including 218 emergency lifesaving centers and 235

emergency departments, to determine the demographic and clinical features and prognosis of acute liver failure patients hospitalized in their respective institutions between 2006 and 2008. Patients with prothrombin time values of 50% or less of the standardized values, or INRs of 1.5 or more, and those with grade II or more severe hepatic encephalopathy were enrolled; however, patients fulfilling the diagnostic criteria for fulminant hepatitis and late-onset hepatic failure (LOHF), a disease related to fulminant hepatitis,[5] were excluded from the database (Table 1). In contrast, patients with underlying chronic liver diseases were included in the evaluation. The patients were classified into five groups; namely, those without hepatic encephalopathy or with grade I hepatic encephalopathy (group 1), those showing grade II or more severe hepatic encephalopathy within 10 days, between 11 and 56 days, or later than 56 days after the onset of symptoms (group 2, group 3 and group 4, respectively), and those with underlying chronic liver diseases (group 5). The nationwide survey was conducted with the approval of the ethics committee of Kagoshima University Graduate School of Medical and Dental Sciences.

Development of diagnostic criteria for "acute liver failure" in Japan

The diagnostic criteria for "acute liver failure" were established based on the results of the following evaluations: (i) the nationwide surveys of fulminant hepatitis and LOHF conducted by the Intractable Liver Diseases Study Group of Japan between 1999 and 2004[1] and by the Intractable Hepato-Biliary Diseases Study Group of Japan between 2005 and 2009;[6–10] (ii) the survey of commercial kits used at institutions to which members of the Japan Society of Hepatology are affiliated; and (iii) the nationwide survey of acute liver failure patients who were not diagnosed as having fulminant hepatitis. The task force of the Intractable Liver Diseases Study Group of Japan prepared the preliminary criteria, and the criteria were completed through discussions among all members of the Study Group.

RESULTS

Commercial kits used for prothrombin time measurement in Japan

RESPONSES TO THE questionnaire were obtained from 359 institutions (79.1%). Sixteen types of commercial kits produced by six industrial companies were used, as shown in Table 2. The most frequently used kit was used in 39% of the institutions (138 institutions), and the kits ranked in the top six places, in terms of the frequency of use, accounted for 92% of the kits used at the institutions. The ISI values of these kits ranged from 0.81 to 2.05, thus, the INRs corre-

Table 2 Commercial kits used at institutions to which active members of the Japan Society of Hepatology are affiliated

Commercial kits	Industrial companies	Number of institutions	ISI	INR corresponding to 40% of the standardized values
A	Q	138	0.95–1.27	1.7
B	Q	50	1.21–1.80	1.9
C	Q	11	0.99–1.10	1.6
D	Q	47	1.44–2.05	1.9
E	Q	24	1.16–1.41	1.9
F	R	35	1.22–1.24	1.98
G	R	3	1.22	ND
H	R	2	1.00–1.02	1.86
I	S	27	0.81–1.00	1.7–1.9
J	S	1	1.8	1.8
K	S	1	1.06	2.1
L	T	3	1.18–1.21	1.7
M	T	1	1.65	2.0
N	T	2	1.07–1.10	1.9
O	U	4	1.73	2.12
P	V	1	1.09	ND

ND, not determined.

sponding to 40% of the standardized values differed markedly among the kits (range, 1.6 and 2.12). When the INRs for the kits ranked in the top six positions were evaluated, the mean INR was found to be 1.86 (range, 1.6 to 1.98).

Acute liver failure patients excluded from the disease entity of fulminant hepatitis in Japan

A total of 217 patients were enrolled from 58 institutions (12.8%), and they were classified as shown in Table 3. Hepatic encephalopathy was absent or grade 1 in 79 patients (group 1: 36.4%). In contrast, grade II or more severe hepatic encephalopathy was present in 94 patients (43.3%), and of these, 58, 34 and two patients, respectively, were classified into group 2 (26.7%), group 3 (15.7%) and group 4 (0.9%). The remaining 44 patients were diagnosed as having acute-on-chronic liver injury and were classified into group 5 (20.3%).

The etiologies of liver damage were viral infection, autoimmune hepatitis, drug allergy-induced liver injury, and indeterminate in 121 patients (55.8%). In the remaining 96 patients (44.2%), the etiologies consisted of those in the exclusion lists for fulminant hepatitis and LOHF. Among these, alcoholic liver injury was the most frequent etiology, noted in 43 patients (19.8%). Circulatory disturbance, malignant cell infiltration, drug toxicity-induced liver injury, postoperative liver injury and metabolic disease were noted as the etiologies in 18 (8.3%), eight (3.7%), eight (3.7%), two (0.9%) and one patient (0.5%), respectively. Most of these patients were classified into either group 1 or group 5.

The prognosis was excellent, especially for the group 1 patients, who showed a survival rate of 78% with conventional medical care. The survival rates, however, were lower for the patients belonging to the other groups, being 35%, 12%, 0% and 21%, respectively, for group 2, group 3, group 4 and group 5. A total of 110 patients died despite conventional medical care, and the causes of death were complications, such as bacterial infection, in 44 patients (40%). Especially, in patients with acute liver failure caused by alcoholic liver injury, multiple organ failure developed frequently, serving as the cause of death. Liver transplantation was performed in 10%, 12% and 50% of the patients in group 2, group 3 and group 4, respectively.

Diagnostic criteria for "acute liver failure" in Japan

The diagnostic criteria for "acute liver failure" in Japan are shown in Table 4. Patients showing prothrombin time values of 40% or less of the standardized values or INRs of 1.5 or more due to severe liver damage within 8 weeks of onset of the symptoms are diagnosed as having "acute liver failure", where the liver function prior to the current onset of liver damage is estimated to be normal. Patients without histological findings of hepatitis, such as those with liver injuries caused by drug toxicity, circulatory disturbance or metabolic disease,

Table 3 Disease groups and causative etiology of acute liver failure in patients not diagnosed as having fulminant hepatitis or late-onset hepatic failure

Groups†	Group-1	Group-2	Group-3	Group 4	Group-5	Total
Number of patients	79	58	34	2	44	217
Etiology‡	Percentage of patients in each type					
Viral	32.9	32.8	32.4	0	25.0	30.9
Drug allergy	5.1	13.8	17.6	50.0	2.3	9.2
Autoimmune	0	8.6	11.8	0	0	4.1
Indeterminate	11.4	8.6	17.6	50.0	4.5	10.6
Unclassified	0	3.4	0	0	0	0.9
Drugs toxicity	5.1	5.2	0	0	2.3	3.7
Alcoholic	17.7	12.1	8.8	0	43.2	19.8
Circulatory disturbance	15.2	5.2	0	0	6.8	8.3
Infiltration of malignancy	3.8	0	2.9	0	9.1	3.8
Metabolic disorders	0	0	2.9	0	0	0.5
Postoperative	0	1.7	0	0	2.3	0.9
Miscellaneous	8.9	8.5	5.9	0	4.5	7.4

†Disease groups of acute liver failure shown in Methods. ‡"Indeterminate" means the etiology was uncertain despite sufficient examinations, and "unclassified" means uncertain etiology due to insufficient examinations.

Table 4 Diagnostic criteria for acute liver failure in Japan (2011)

Patients showing prothrombin time values of 40% or less of the standardized values, or international normalized ratios (INRs) of 1.5 or more due to severe liver damage within 8 weeks of the onset of disease symptoms are diagnosed as having "acute liver failure", where the liver function prior to the current onset of liver damage is estimated to be normal based on blood laboratory data and imaging examinations. "Acute liver failure" is classified into "acute liver failure without hepatic coma" and "acute liver failure with hepatic coma"; no or grade I hepatic encephalopathy is present in the former type, while grade II or more severe hepatic encephalopathy is found in the latter type. "Acute liver failure with hepatic coma" is further subclassified into two disease types; the "acute type" and "subacute type", respectively, with grade II or more severe hepatic encephalopathy developing within 10 days or between 11 and 56 days after the onset of disease symptoms, respectively, in the two types.

Note 1: Hepatitis B virus (HBV) carriers and autoimmune hepatitis patients showing acute exacerbation of hepatitis in the normal liver are included under the disease entity of "acute liver failure". In the case of indeterminate previous liver function, the patients with both etiologies are diagnosed as having "acute liver failure" when no liver function impairment preceding the exacerbation of the liver injury can be confirmed.

Note 2: In general, alcoholic hepatitis develops in patients with chronic liver diseases caused by habitual alcohol consumption. Thus, patients with alcoholic hepatitis are excluded from the disease entity of "acute liver failure". However, patients with fatty liver caused by alcohol intake or metabolic syndrome, including obesity, are diagnosed as having "acute liver failure" if etiologies other than habitual alcohol consumption are responsible for the acute injury in the liver, in the absence of prior impairment of liver function.

Note 3: Patients without histological evidence of hepatitis, such as inflammatory lymphocytic infiltration, are included under the disease entity of "acute liver failure". Thus, patients with liver damage caused by drug toxicity, circulatory disturbance or metabolic disease and acute fatty liver of pregnancy are diagnosed as having "acute liver failure," while they are excluded from the disease entity of "fulminant hepatitis". In contrast, patients with liver injury caused by viral infection, autoimmune hepatitis and drug allergy-induced hepatitis are included under the disease entities of "fulminant hepatitis" as well as "acute liver failure".

Note 4: The severity of hepatic encephalopathy is diagnosed depending on the classification presented in the Imuyama Symposium in 1972 (Table 5). Also, hepatic encephalopathy developing in pediatric and infantile patients is classified according to the criteria proposed by the 5th Workshop on Pediatric Liver Diseases in 1988 (Table 6).

Note 5: The etiology of "acute liver failure" is classified according to the criteria proposed by the Intractable Liver Diseases Study Group of Japan at 2002, with some modifications (Table 7).

Note 6: Patients showing prothrombin time values of less than 40% of the standardized values or INRs of 1.5 or more and grade II or more severe hepatic coma between 8 and 24 weeks of the onset of disease symptoms are diagnosed as having late-onset hepatic failure (LOHF), as a disease related to "acute liver failure".

are also included in the disease entity of "acute liver failure", while acute-on-chronic liver injuries, such as liver injury caused by alcohol, are excluded. Acute liver failure patients are classified into those with and without hepatic coma, depending on the severity of the hepatic encephalopathy, and "acute liver failure with hepatic coma" is further subclassified into two disease types, the "acute type" and the "subacute type". To clarify the concept of "acute liver failure" in Japan, five items are described in the footnote of the diagnostic criteria.

DISCUSSION

TO ESTABLISH THE diagnostic criteria for "acute liver failure" in Japan, two types of nationwide surveys were performed; the survey of commercial kits used for measurement of the prothrombin time used at institutions to which hepatology specialists were affiliated and the survey of acute liver failure patients who were excluded from the disease entities of fulminant hepatitis and LOHF. The former survey revealed that 16 commercial kits were used, and the INRs corresponding to 40% of the standardized values differed among the kits (range, 1.6 and 2.12), suggesting that an INR of 1.5 or more was a suitable cutoff value to include all patients previously diagnosed as having fulminant hepatitis or LOHF in the novel disease entity of "acute liver failure". On the other hand, the latter survey demonstrated that patients with acetaminophen-induced toxic liver injury, the most frequent cause of acute liver failure in Europe and the United States, were seldom found in Japan. In contrast, alcoholic liver injury was observed frequently among patients showing prolongation of the prothrombin time and/or grade II or more severe hepatic encephalopathy. The clinical features of these patients,