2). In patients with cirrhosis, BCAAs increase liver-associated lymphocyte counts and restore phagocytic function of neutrophils and natural killer activity of lymphocytes.[51] Moreover, BCAA treatment may suppress hepatic oxidative stress by modulating the redox state of albumin.[52,53] Serum albumin is divided into two forms, reduced and oxidized albumin, depending on the redox state at Cys34,[54,55] and the oxidized/reduced albumin ratio increases in patients with cirrhosis.[56,57] BCAA supplementation increases ratio of reduced albumin[52] and decreases iron-related oxidative stress in patients with cirrhosis,[53] suggesting that BCAAs may reduce the iron-induced oxidative stress through a qualitative alteration of serum albumin. Thus, BCAAs may suppress hepatocarcinogenesis partly by improvement of immune function and reduction of oxidative stress.

Mortality and Clinical Decompensation

Some reports suggest that oral BCAA supplementation improves survival in a rat model of cirrhosis and in decompensated patients with cirrhosis.[58-60] Marchesini et al. first performed a randomized, controlled trial exploring the usefulness of BCAAs in patients with cirrhosis.[15] One year of BCAA treatment significantly reduced the occurrence of the primary outcome (a composite of death, number of hospital admissions, and duration of hospital stay) compared to that in the lactalbumin-treated group.[15] Although this study shows the effectiveness of BCAA supplementation, the complications that contributed to the reduction of outcome incidence was not identified because of a small number of enrolled patients (n = 59 in BCAA group) and high dropout rate (15% in the BCAA group) due to poor compliance with the BCAA supplement.

Since 1996, a BCAA supplement formulation (L-Val:L-Leu:L-Ile = 1.2:2:1; Ajinomoto Pharmaceuticals, Tokyo, Japan) has been approved for use in cirrhosis in Japan. The supplement is in the form of small uniform granules, which reduces BCAA-induced stimulation of taste buds and contributes to improved compliance. Using these BCAA granules, Muto et al. performed a large (n = 314 in the BCAA group) randomized, controlled trial.[16] None of the patients discontinued the study because of poor compliance. A preplanned safety analysis revealed that BCAA granules significantly reduced the occurrence of the overall primary outcome (hepatic failure, variceal bleeding, development of liver cancer, and death from any cause) compared to that in the control diet group. Among individual events of primary outcome, the occurrence of hepatic failure was significantly less in the BCAA group compared to the control diet group (hazard ratio 0.45; 95% confidence interval 0.23-0.88; $P = 0.016$). On the basis of the results, the Data and Safety Monitoring Board concluded that the harm associated with the increased occurrence of primary outcome in the control diet group outweigh any potential benefits and the study was discontinued 10 months early due to safety concerns. Beneficial effects of BCAAs on clinical decompensation, including development of hepatic failure, are also reported in patients with cirrhosis accompanied with HCC.[61-63] Thus, the treatment with BCAA supplementation is now recommended in the guidelines for the treatment of liver cirrhosis by the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis from the Ministry of Health, Labour and Welfare of Japan.[64]

Quality of Life

Generally, the overall health status and QOL of patients with liver cirrhosis is poor.[65,66] Patients with cirrhosis frequently complain of fatigue and sleep disturbances. There is, however, no standard approach to the management of these symptoms in the absence of overt hepatic encephalopathy.[67] In a randomized study, BCAA-enriched supplements have been reported to improve weakness and easy fatigability compared to ordinary food.[20] BCAA-enriched supplementation has also been reported to improve the Epworth Sleepiness Scale score.[21] In large-scale randomized controlled trials, BCAA supplementation was found to significantly improve the Short Form-36 scores of general health perception compared to control groups.[15,16]

Although it is still unclear how BCAA supplementation provides relief from fatigue and sleep disturbances in patients with cirrhosis, there are at least three possible mechanisms. First, fatigue and sleep disturbances could be caused by minimal hepatic encephalopathy, and BCAA may ameliorate these symptoms by improving this condition.[68] Second, increased serum tryptophan levels are known to impair the QOL in various conditions involving malnourishment, including liver cirrhosis.[69] Tryptophan is a precursor for the neurotransmitter 5-hydroxytryptamine, which is associated with fatigue and sleep disturbances.[70] Because BCAAs compete with tryptophan for transport into the brain, these symptoms may be alleviated by supplementation with BCAAs.[71] Third, impaired cerebral blood flow is associated with fatigue and sleep disturbance[72] and is decreased in patients with liver cirrhosis.[73,74] BCAA supplementation is known to improve cerebral blood flow, possibly resulting in lessened fatigue and sleep disturbances.[75,76]

Muscle cramps are also associated with poor QOL in patients with liver cirrhosis,[77] and the frequency of muscle cramps has been reported to be dramatically reduced by BCAA supplementation over a period of 3 months (7.4 ± 2.0 versus 0.3 ± 0.5 times/week).[78] Muscle cramps are caused by a variety of factors, including diuretic treatment, reduction of circulating volume, and deficiency of vitamin E and taurine.[79] Amino acid imbalance decreases taurine production, and therefore, BCAA may inhibit muscle cramps, possibly through improvement of the imbalance and consequent restoration of taurine production.[78,79]

Conclusion

In this article, we have reviewed evidence for potential pharmaceutical properties of BCAAs on various physiological and clinical events associated with chronic liver disease. Evidence for beneficial effects of BCAA supplementation has yet to be fully validated, and improvement for low compliance of BCAA supplementation is still required. However, there is substantial evidence that depletion of serum BCAA levels is involved in the progression of liver disease and the development of clinically important sequelae. Pharmacological supplementation with BCAAs may be a promising therapeutic strategy for patients with liver cirrhosis.

References

1. Charlton MR. Protein metabolism and liver disease. Baillieres Clin Endocrinol Metab 1996;10:617-635.
2. Kawaguchi T, Yamagishi S, Sata M. Branched-chain amino acids and pigment epithelium-derived factor: novel therapeutic agents for hepatitis c virus-associated insulin resistance. Curr Med Chem 2009;16: 4843-4857.
3. Yamato M, Muto Y, Yoshida T, Kato M, Moriwaki M. Clearance rate of plasma branched-chain amino acids correlates significantly with blood ammonia level in patients with liver cirrhosis. Int Hepatol Commun 1995;3:91-96.
4. Okuno M, Moriwaki H, Kato M, Muto Y, Kojima S. Changes in the ratio of branched-chain to aromatic amino acids affect the secretion of albumin in cultured rat hepatocytes. Biochem Biophys Res Commun 1995;214:1045-1050.
5. Steigmann F, Szanto PB, Poulos A, Lim PE, Dubin A. Significance of serum aminograms in diagnosis and prognosis of liver diseases. J Clin Gastroenterol 1984;6:453-460.
6. Plauth M, Egberts EH, Hamster W, Torok M, Muller PH, Brand O, et al. Long-term treatment of latent portosystemic encephalopathy with branched-chain amino acids. A double-blind placebo-controlled crossover study. J Hepatol 1993;17:308-314.
7. Keshavarzian A, Meek J, Sutton C, Emery VM, Hughes EA, Hodgson HJ. Dietary protein supplementation from vegetable sources in the management of chronic portal systemic encephalopathy. Am J Gastroenterol 1984;79:945-949.
8. ASPEN Board of Directors and the Clinical Guidelines Task Force. Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. JPEN J Parenter Enteral Nutr 2002;26(suppl 1): 1SA-138SA.
9. Plauth M, Cabre E, Riggio O, Assis-Camilo M, Pirlich M, Kondrup J, et al. ESPEN Guidelines on Enteral Nutrition: Liver disease. Clin Nutr 2006;25:285-294.
10. Suzuki K, Suzuki K, Koizumi K, Ichimura H, Oka S, Takada H, et al. Measurement of serum branched-chain amino acids to tyrosine ratio level is useful in a prediction of a change of serum albumin level in chronic liver disease. Hepatol Res 2008;38:267-272.
11. Fischer JE, Funovics JM, Aguirre A, James JH, Keane JM, Wesdorp RI, et al. The role of plasma amino acids in hepatic encephalopathy. Surgery 1975;78:276-290.
12. Ijichi C, Matsumura T, Tsuji T, Eto Y. Branched-chain amino acids promote albumin synthesis in rat primary hepatocytes through the mTOR signal transduction system. Biochem Biophys Res Commun 2003;303:59-64.
13. Nishitani S, Takehana K, Fujitani S, Sonaka I. Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. Am J Physiol Gastrointest Liver Physiol 2005;288:G1292-G1300.
14. She P, Reid TM, Bronson SK, Vary TC, Hajnal A, Lynch CJ, et al. Disruption of BCATm in mice leads to increased energy expenditure associated with the activation of a futile protein turnover cycle. Cell Metab 2007;6:181-194.
15. Marchesini G, Bianchi G, Merli M, Amodio P, Panella C, Loguercio C, et al. Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. Gastroenterology 2003;124:1792-1801.
16. Muto Y, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, et al. Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. Clin Gastroenterol Hepatol 2005;3: 705-713.
17. Muto Y, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, et al. Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. Hepatol Res 2006;35:204-214.
18. Kawaguchi T, Nagao Y, Matsuoka H, Ide T, Sata M. Branched-chain amino acid-enriched supplementation improves insulin resistance in patients with chronic liver disease. Int J Mol Med 2008;22:105-112.
19. Kakazu E, Ueno Y, Kondo Y, Fukushima K, Shiina M, Inoue J, et al. Branched chain amino acids enhance the maturation and function of myeloid dendritic cells ex vivo in patients with advanced cirrhosis. HEPATOLOGY 2009;50:1936-1945.
20. Nakaya Y, Okita K, Suzuki K, Moriwaki H, Kato A, Miwa Y, et al. BCAA-enriched snack improves nutritional state of cirrhosis. Nutrition 2007;23:113-120.
21. Ichikawa T, Naota T, Miyaaki H, Miuma S, Isomoto H, Takeshima F, et al. Effect of an oral branched chain amino acid-enriched snack in cirrhotic patients with sleep disturbance. Hepatol Res 2010;40: 971-978.
22. Charlton M. Branched-chain amino acid enriched supplements as therapy for liver disease. J Nutr 2006;136(suppl 1):295S-298S.
23. Moriwaki H, Miwa Y, Tajika M, Kato M, Fukushima H, Shiraki M. Branched-chain amino acids as a protein- and energy-source in liver cirrhosis. Biochem Biophys Res Commun 2004;313:405-409.
24. Montoya A, Gomez-Lechon MJ, Castell JV. Influence of branched-chain amino acid composition of culture media on the synthesis of plasma proteins by serum-free cultured rat hepatocytes. In Vitro Cell Dev Biol 1989;25:358-364.
25. Kuwahata M, Yoshimura T, Sawai Y, Amano S, Tomoe Y, Segawa H, et al. Localization of polypyrimidine-tract-binding protein is involved in the regulation of albumin synthesis by branched-chain amino acids in HepG2 cells. J Nutr Biochem 2008;19:438-447.
26. Nishitani S, Ijichi C, Takehana K, Fujitani S, Sonaka I. Pharmacological activities of branched-chain amino acids: specificity of tissue and signal transduction. Biochem Biophys Res Commun 2004;313: 387-389.

27. Kawamura-Yasui N, Kaito M, Nakagawa N, Fujita N, Ikoma J, Gabazza EC, et al. Evaluating response to nutritional therapy using the branched-chain amino acid/tyrosine ratio in patients with chronic liver disease. J Clin Lab Anal 1999;13:31-34.
28. Layman DK, Shiue H, Sather C, Erickson DJ, Baum J. Increased dietary protein modifies glucose and insulin homeostasis in adult women during weight loss. J Nutr 2003;133:405-410.
29. Zhang Y, Guo K, LeBlanc RE, Loh D, Schwartz GJ, Yu YH. Increasing dietary leucine intake reduces diet-induced obesity and improves glucose and cholesterol metabolism in mice via multimechanisms. Diabetes 2007;56:1647-1654.
30. Ikehara O, Kawasaki N, Maezono K, Komatsu M, Konishi A. Acute and chronic treatment of L-isoleucine ameliorates glucose metabolism in glucose-intolerant and diabetic mice. Biol Pharm Bull 2008;31: 469-472.
31. Nishitani S, Matsumura T, Fujitani S, Sonaka I, Miura Y, Yagasaki K. Leucine promotes glucose uptake in skeletal muscles of rats. Biochem Biophys Res Commun 2002;299:693-696.
32. Hinault C, Mothe-Satney I, Gautier N, Lawrence JC Jr, Van Obberghen E. Amino acids and leucine allow insulin activation of the PKB/mTOR pathway in normal adipocytes treated with wortmannin and in adipocytes from db/db mice. FASEB J 2004;18:1894-1896.
33. Higuchi N, Kato M, Miyazaki M, Tanaka M, Kohjima M, Ito T, et al. Potential role of branched-chain amino acids in glucose metabolism through the accelerated induction of the glucose-sensing apparatus in the liver. J Cell Biochem 2011;112:30-38.
34. Nishimura J, Masaki T, Arakawa M, Seike M, Yoshimatsu H. Isoleucine prevents the accumulation of tissue triglycerides and upregulates the expression of PPARalpha and uncoupling protein in diet-induced obese mice. J Nutr 2010;140:496-500.
35. Arakawa M, Masaki T, Nishimura J, Seike M, Yoshimatsu H. The effects of branched-chain amino acid granules on the accumulation of tissue triglycerides and uncoupling proteins in diet-induced obese mice. Endocr J 2011;58:161-170.
36. Tabaru A, Shirohara H, Moriyama A, Otsuki M. Effects of branched-chain-enriched amino acid solution on insulin and glucagon secretion and blood glucose level in liver cirrhosis. Scand J Gastroenterol 1998; 33:853-859.
37. Korenaga K, Korenaga M, Uchida K, Yamasaki T, Sakaida I. Effects of a late evening snack combined with alpha-glucosidase inhibitor on liver cirrhosis. Hepatol Res 2008;38:1087-1097.
38. Sakaida I, Tsuchiya M, Okamoto M, Okita K. Late evening snack and the change of blood glucose level in patients with liver cirrhosis. Hepatol Res 2004;30S:67-72.
39. Kawaguchi T, Taniguchi E, Itou M, Sumie S, Oriishi T, Matsuoka H, et al. Branched-chain amino acids improve insulin resistance in patients with hepatitis C virus-related liver disease: report of two cases. Liver Int 2007;27:1287-1292.
40. Tsuchiya K, Asahina Y, Izumi N. Long time oral supplementation with branched-chain amino acids improves survival and decreases recurrences in patients with hepatocellular carcinoma [in Japanese]. Nippon Shokakibyo Gakkai Zasshi 2008;105:808-816.
41. Iwasa J, Shimizu M, Shiraki M, Shirakami Y, Sakai H, Terakura Y, et al. Dietary supplementation with branched-chain amino acids suppresses diethylnitrosamine-induced liver tumorigenesis in obese and diabetic C57BL/KsJ-db/db mice. Cancer Sci 2010;101:460-467.
42. Yoshiji H, Noguchi R, Kaji K, Ikenaka Y, Shirai Y, Namisaki T, et al. Attenuation of insulin-resistance-based hepatocarcinogenesis and angiogenesis by combined treatment with branched-chain amino acids and angiotensin-converting enzyme inhibitor in obese diabetic rats. J Gastroenterol 2010;45:443-450.
43. Paradis V, Zalinski S, Chelbi E, Guedj N, Degos F, Vilgrain V, et al. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. HEPATOLOGY 2009;49:851-859.
44. Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, et al. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol 2004;165:1499-1508.
45. Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. HEPATOLOGY 2010;51:1820-1832.
46. Formisano P, Oriente F, Fiory F, Caruso M, Miele C, Maitan MA, et al. Insulin-activated protein kinase Cbeta bypasses Ras and stimulates mitogen-activated protein kinase activity and cell proliferation in muscle cells. Mol Cell Biol 2000;20:6323-6333.
47. Sandhu MS, Dunger DB, Giovannucci EL. Insulin, insulin-like growth factor-I (IGF-I), IGF binding proteins, their biologic interactions, and colorectal cancer. J Natl Cancer Inst 2002;94:972-980.
48. Scharf JG, Dombrowski F, Ramadori G. The IGF axis and hepatocarcinogenesis. Mol Pathol 2001;54:138-144.
49. Qi HL, Zhang Y, Ma J, Guo P, Zhang XY, Chen HL. Insulin/protein kinase B signalling pathway upregulates metastasis-related phenotypes and molecules in H7721 human hepatocarcinoma cell line. Eur J Biochem 2003;270:3795-3805.
50. Nitta T, Kim JS, Mohuczy D, Behrns KE. Murine cirrhosis induces hepatocyte epithelial mesenchymal transition and alterations in survival signaling pathways. HEPATOLOGY 2008;48:909-919.
51. Nakamura I, Ochiai K, Imai Y, Moriyasu F, Imawari M. Restoration of innate host defense responses by oral supplementation of branched-chain amino acids in decompensated cirrhotic patients. Hepatol Res 2007;37:1062-1067.
52. Fukushima H, Miwa Y, Shiraki M, Gomi I, Toda K, Kuriyama S, et al. Oral branched-chain amino acid supplementation improves the oxidized/reduced albumin ratio in patients with liver cirrhosis. Hepatol Res 2007;37:765-770.
53. Ohno T, Tanaka Y, Sugauchi F, Orito E, Hasegawa I, Nukaya H, et al. Suppressive effect of oral administration of branched-chain amino acid granules on oxidative stress and inflammation in HCV-positive patients with liver cirrhosis. Hepatol Res 2008;38:683-688.
54. Halliwell B, Gutteridge JM. The antioxidants of human extracellular fluids. Arch Biochem Biophys 1990;280:1-8.
55. Hayashi T, Suda K, Imai H, Era S. Simple and sensitive high-performance liquid chromatographic method for the investigation of dynamic changes in the redox state of rat serum albumin. J Chromatogr B Analyt Technol Biomed Life Sci 2002;772:139-146.
56. Watanabe A, Matsuzaki S, Moriwaki H, Suzuki K, Nishiguchi S. Problems in serum albumin measurement and clinical significance of albumin microheterogeneity in cirrhotics. Nutrition 2004;20:351-357.
57. Sakata M, Kawaguchi T, Taniguchi E, Nakayama A, Ishizaki S, Sonaka I, et al. Oxidized albumin is associated with water retention and severity of disease in patients with chronic liver diseases. e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism 2010;5: e247-e253.
58. Ichida T, Shibasaki K, Muto Y, Satoh S, Watanabe A, Ichida F. Clinical study of an enteral branched-chain amino acid solution in decompensated liver cirrhosis with hepatic encephalopathy. Nutrition 1995; 11(suppl 2):238-244.
59. Kajiwara K, Okuno M, Kobayashi T, Honma N, Maki T, Kato M, et al. Oral supplementation with branched-chain amino acids improves survival rate of rats with carbon tetrachloride-induced liver cirrhosis. Dig Dis Sci 1998;43:1572-1579.
60. Yoshida T, Muto Y, Moriwaki H, Yamato M. Effect of long-term oral supplementation with branched-chain amino acid granules on the prognosis of liver cirrhosis. Gastroenterol Jpn 1989;24:692-698.
61. Kuroda H, Ushio A, Miyamoto Y, Sawara K, Oikawa K, Kasai K, et al. Effects of branched-chain amino acid-enriched nutrient for patients with hepatocellular carcinoma following radiofrequency ablation: a one-year prospective trial. J Gastroenterol Hepatol 2010;25: 1550-1555.
62. Meng WC, Leung KL, Ho RL, Leung TW, Lau WY. Prospective randomized control study on the effect of branched-chain amino acids in patients with liver resection for hepatocellular carcinoma. Aust N Z J Surg 1999;69:811-815.

63. Poon RT, Yu WC, Fan ST, Wong J. Long-term oral branched chain amino acids in patients undergoing chemoembolization for hepatocellular carcinoma: a randomized trial. Aliment Pharmacol Ther 2004;19: 779-788.
64. Kumada H, Okanoue T, Onji M, Moriwaki H, Izumi N, Tanaka E, et al. Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. Hepatol Res 2010;40:8-13.
65. Gross CR, Malinchoc M, Kim WR, Evans RW, Wiesner RH, Petz JL, et al. Quality of life before and after liver transplantation for cholestatic liver disease. HEPATOLOGY 1999;29:356-364.
66. Kawamura N, Nakajima H, Takashi SI. Administration of granulated BCAA and quality of life. Hepatol Res 2004;30S:42-45.
67. Cordoba J, Cabrera J, Lataif L, Penev P, Zee P, Blei AT. High prevalence of sleep disturbance in cirrhosis. HEPATOLOGY 1998;27: 339-345.
68. Wilkinson DJ, Smeeton NJ, Watt PW. Ammonia metabolism, the brain and fatigue; revisiting the link. Prog Neurobiol 2010;91: 200-219.
69. Huang A, Fuchs D, Widner B, Glover C, Henderson DC, Allen-Mersh TG. Serum tryptophan decrease correlates with immune activation and impaired quality of life in colorectal cancer. Br J Cancer 2002;86: 1691-1696.
70. Davis JM, Alderson NL, Welsh RS. Serotonin and central nervous system fatigue: nutritional considerations. Am J Clin Nutr 2000;72(suppl 2):573S-578S.
71. Fernstrom JD. Branched-chain amino acids and brain function. J Nutr 2005;135(suppl 6):1539S-1546S.
72. Biswal B, Kunwar P, Natelson BH. Cerebral blood flow is reduced in chronic fatigue syndrome as assessed by arterial spin labeling. J Neurol Sci 2011;301:9-11.
73. Ahl B, Weissenborn K, van den Hoff J, Fischer-Wasels D, Kostler H, Hecker H, et al. Regional differences in cerebral blood flow and cerebral ammonia metabolism in patients with cirrhosis. HEPATOLOGY 2004;40:73-79.
74. Iwasa M, Matsumura K, Kaito M, Ikoma J, Kobayashi Y, Nakagawa N, et al. Decrease of regional cerebral blood flow in liver cirrhosis. Eur J Gastroenterol Hepatol 2000;12:1001-1006.
75. Iwasa M, Matsumura K, Watanabe Y, Yamamoto M, Kaito M, Ikoma J, et al. Improvement of regional cerebral blood flow after treatment with branched-chain amino acid solutions in patients with cirrhosis. Eur J Gastroenterol Hepatol 2003;15:733-737.
76. Yamamoto M, Iwasa M, Matsumura K, Nakagawa Y, Fujita N, Kobayashi Y, et al. Improvement of regional cerebral blood flow after oral intake of branched-chain amino acids in patients with cirrhosis. World J Gastroenterol 2005;11:6792-6799.
77. Marchesini G, Bianchi G, Amodio P, Salerno F, Merli M, Panella C, et al. Factors associated with poor health-related quality of life of patients with cirrhosis. Gastroenterology 2001;120:170-178.
78. Sako K, Imamura Y, Nishimata H, Tahara K, Kubozono O, Tsubouchi H. Branched-chain amino acids supplements in the late evening decrease the frequency of muscle cramps with advanced hepatic cirrhosis. Hepatol Res 2003;26:327-329.
79. Corbani A, Manousou P, Calvaruso V, Xirouchakis I, Burroughs AK. Muscle cramps in cirrhosis: the therapeutic value of quinine. Is it underused? Dig Liver Dis 2008;40:794-799.

BMC Medical Genetics

RESEARCH ARTICLE Open Access

No association for Chinese HBV-related hepatocellular carcinoma susceptibility SNP in other East Asian populations

Hiromi Sawai[1*], Nao Nishida[1,2], Hamdi Mbarek[3], Koichi Matsuda[3], Yoriko Mawatari[2], Megumi Yamaoka[1], Shuhei Hige[4], Jong-Hon Kang[5], Koichi Abe[6], Satoshi Mochida[7], Masaaki Watanabe[8], Masayuki Kurosaki[9], Yasuhiro Asahina[9], Namiki Izumi[9], Masao Honda[10], Shuichi Kaneko[10], Eiji Tanaka[11], Kentaro Matsuura[12], Yoshito Itoh[13], Eiji Mita[14], Masaaki Korenaga[15], Keisuke Hino[15], Yoshikazu Murawaki[16], Yoichi Hiasa[17], Tatsuya Ide[18], Kiyoaki Ito[2], Masaya Sugiyama[2], Sang Hoon Ahn[19], Kwang-Hyub Han[19], Jun Yong Park[19], Man-Fung Yuen[20], Yusuke Nakamura[3], Yasuhito Tanaka[12], Masashi Mizokami[2] and Katsushi Tokunaga[1]

Abstract

Background: A recent genome-wide association study (GWAS) using chronic HBV (hepatitis B virus) carriers with and without hepatocellular carcinoma (HCC) in five independent Chinese populations found that one SNP (rs17401966) in *KIF1B* was associated with susceptibility to HCC. In the present study, a total of 580 HBV-derived HCC cases and 1351 individuals with chronic hepatitis B (CHB) or asymptomatic carrier (ASC) were used for replication studies in order to evaluate the reported association with HBV-derived HCC in other East Asian populations.

Results: We did not detect any associations between rs17401966 and HCC in the Japanese cohorts (replication 1: OR = 1.09, 95 % CI = 0.82-1.43; replication 2: OR = 0.79, 95 % CI = 0.54-1.15), in the Korean cohort (replication 3: OR = 0.95, 95 % CI = 0.66-1.36), or in the Hong Kong Chinese cohort (replication 4: OR = 1.17, 95 % CI = 0.79-1.75). Meta-analysis using these cohorts also did not show any associations with P = 0.97.

Conclusions: None of the replication cohorts showed associations between rs17401966 and HBV-derived HCC. This may be due to differences in the genetic diversity among the Japanese, Korean and Chinese populations. Other reasons could be the high complexity of multivariate interactions between the genomic information and the phenotype that is manifesting. A much wider range of investigations is needed in order to elucidate the differences in HCC susceptibility among these Asian populations.

Keywords: Hepatitis B, hepatocellular carcinoma, candidate SNP, replication study, genome-wide association study

Background

Hepatitis B (HB) is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV), and approximately 360 million people worldwide are thought to be chronically infected with HBV. The clinical course of HBV infection is variable, including acute self-limiting infection, fulminant hepatic failure, inactive carrier state and chronic hepatitis with progression to cirrhosis and hepatocellular carcinoma (HCC). Although some HBV carriers spontaneously eliminate the virus, 2-10 % of individuals with chronic HB (CHB) develop liver cirrhosis every year, and a subset of these individuals suffer from liver failure or HCC. Around 600,000 new HCC cases are diagnosed annually worldwide, with HCC being relatively common in Asia-Pacific countries and sub-Saharan Africa; more than 70 % of HCC patients are diagnosed in Asia (with 55 % in China) [1]. However, HCC is relatively uncommon in the USA, Europe and Australia [1,2]. The majority of HCC develops in patients with cirrhosis, which is most often attributable

* Correspondence: sawai@m.u-tokyo.ac.jp
[1]Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
Full list of author information is available at the end of the article

BioMed Central

© 2012 Sawai et al.; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

to chronic HBV infection followed by chronic HCV in the Asia-Pacific region [3].

A recent genome-wide association study (GWAS) using Japanese CHB cases and controls confirmed that 11 SNPs in a region including *HLA-DPA1* and *-DPB1* were associated with CHB [4]. Moreover, a GWAS using chronic HBV carriers with and without HCC in five independent Chinese populations reported that one SNP (rs17401966) in *KIF1B* was associated with HCC susceptibility [5]. In the present study, we performed replication studies using Japanese, Korean and Hong Kong Chinese cases and controls in order to evaluate the reported association with HBV-derived HCC in other East Asian populations.

Results

We performed SNP genotyping of rs17401966 located in the *KIF1B* gene for the purpose of replication analysis of the previous GWAS report [5]. Four distinct cohorts were used for these replication analyses (Table 1). We first examined two independent Japanese case–control samples including 179 cases and 769 controls from Biobank Japan (replication 1), and 142 cases and 251 controls from various hospitals (replication 2). We did not detect any associations between rs17401966 and HCC in the Japanese cohorts (replication 1: OR = 1.09; 95 % CI = 0.82-1.43, replication 2: OR = 0.79; 95 % CI = 0.54-1.15). We further examined Korean case–control samples comprising 164 cases and 144 controls (replication 3) and Hongkongese 94 HCC cases and 187 CHB controls (replication 4), but again did not detect any association (replication 3: OR = 0.95; 95 % CI = 0.66-1.36, replication 4: OR = 1.17; 95 % CI = 0.79-1.75). Logistic regression analysis adjusted for age and gender also did not show any association (P_{log} = 0.65, 0.27, 0.11, 0.56 for each replication panel). Moreover, we conducted meta-analysis to combine these studies, also not detect any association (P_{meta} = 0.97).

Discussion and conclusions

Zhang et al. [5] reported that SNP rs17401966 was significantly associated with HBV-related HCC (joint OR = 0.61). They conducted a GWAS using 348 cases and 359 controls in a population in Guangxi in southern China, and selected 45 SNPs for the replication study based on the results ($P < 10^{-4}$). In the first replication study, they used 276 cases and 266 controls from Beijing in northern China, and 5 SNPs showed the same direction of association as in the GWAS ($P < 0.05$). They performed a further replication study (of 507 cases and 215 controls) in Jiangsu in eastern China and only one SNP showed the same trend ($P = 3.9 \times 10^{-5}$). Guangdong and Shanghai samples from southern and eastern China were used for further replication studies. The association yielded a p-value of 1.7×10^{-18} on meta-analysis.

We performed four replication analyses using Japanese, Korean and Hong Kong Chinese samples (Table 1). Although sample size of each cohort is smaller than that of the previous GWAS, we conducted mete-analysis of all our study. The result did not show any association between rs17401966 and HBV-derived HCC (P_{meta} = 0.97).

This may be due to differences in genetic diversity among Japanese, Korean and Chinese populations. A maximum-likelihood tree of 126 populations based on 19,934 SNPs showed that Japanese and Korean populations form a monophyletic clade with a 100 % bootstrap value [6]. However, Chinese populations form a paraphyletic clade with two other populations. This indicates that Japanese and Korean populations are genetically closer to one another than the Chinese population.

Table 1 Association between rs17401966 and HBV-derived HCC

cohort	sample size	cases			controls				OR		
	(cases/controls)	GG	AG	AA	GG	AG	AA	HWE p	(95 % CI)	P [a]	P_{het} [b]
replication 1	179/769	13	61	105	45	261	463	0.599	1.09	0.578	
(Japan 1)		(7.2)	(34.1)	(58.7)	(5.9)	(33.9)	(60.2)		(0.82-1.43)		
replication 2	142/251	5	46	91	14	91	146	1	0.79	0.212	
(Japan 2)		(3.5)	(32.4)	(64.1)	(5.6)	(36.2)	(58.2)		(0.54-1.15)		
replication 3	164/144	17	59	88	15	55	74	0.616	0.95	0.790	
(Korea)		(10.4)	(36.0)	(53.6)	(10.4)	(38.2)	(51.4)		(0.66-1.36)		
replication 4	94/187	10	39	44	13	80	94	0.767	1.17	0.432	
(Hong Kong)		(10.6)	(41.5)	(46.8)	(6.9)	(42.8)	(50.3)		(0.79-1.75)		
Meta-analysis [c]									0.996	0.965	0.423
									(0.84-1.18)		

[a]P value of fisher's exact test for allele model.
[b]Result of Breslow-Day test.
[c]Results of meta-analysis were calculated by the Mantel-Haenzel method.

We did not find any association with Hong Kong Chinese cohort (P = 0.43). Moreover, a study using 357 HCC cases and 354 HBV-positive non-HCC controls in Hong Kong Chinese did not show any significant difference (P = 0.91) [7]. Previous population studies have revealed that various Han Chinese populations show varying degrees of admixture between a northern Altaic cluster and a southern cluster of Sino-Tibetan/Tai-Kadai populations in southern China and northern Thailand [6]. Although Hong Kong is located closed to the Guangdong (cohort 3 of Zhang et al study), there is great heterogeneity for rs17401966 between Hong Kong cohorts (our study and Chan's study [7]) and Guangdong cohort (our study versus Zhang's study: $P_{het} = 0.0066$; Chan's study versus Zhang's study: $P_{het} = 0.035$). This result suggests the existence of other confounding factors, which can differentiate the previous study in China and this study.

One of the possible reasons could be the high complexity of multivariate interactions between the genomic information and the phenotype that is manifesting. HCC development is a multiple process which links to causative factors such as age, gender, environmental toxins, alcohol and drug abuse, higher HBV DNA levels, and HBV genotype variations [8]. The eight HBV genotypes display distinct geographical and ethnic distributions. Genotypes B and C are prevalent in Asia. Specific variations in HBV have been associated with cirrhosis and HCC. These variations include in particular mutations in pre-core region (Pre-C), in basal core promoter (BCP) and in ORF encoding Pre-S1/Pre-S2/S and Pre-C/C. Because there is an overlap between Pre-C or BCP mutations and genotypes, these mutations appear to be more common in genotype C as compared to other genotypes [9].

Aflatoxins are a group of 20 related metabolites and Aflatoxin B1 is the most potent naturally occurring chemical liver carcinogen known. Aflatoxin exposures multiplicatively increase the risk of HCC in people chronically infected with HBV, which illustrates the deleterious impact that even low toxin levels in the diet can have on human health [10–12]. Liu and Wu estimated population risk for aflatoxin-induced HCC around the world [13]. Most cases occur in sub-Saharan Africa, Southeast Asia and China, where populations suffer from both high HBV prevalence and largely uncontrolled exposure to aflatoxin in food. But we could not obtain the information of these confounding factors from both of the previous GWAS study and this study. A much wider range of investigations is thus needed in order to elucidate the differences in HCC susceptibility among these Asian populations.

Methods

Samples

Case and control samples used in this study were collected from Japan, Korea and Hong Kong listed in supplementary Additional file 1: Table S1. A total of 179 cases and 769 control subjects were analyzed in the first replication study. DNA samples from both CHB controls and HBV-related HCC cases used in this study were obtained from the BioBank Japan at the Institute of Medical Science, the University of Tokyo [14]. Among the BioBank Japan samples, we selected HBsAg-seropositive CHB patients with elevated serum aminotransferase levels for more than six months, according to the guidelines for diagnosis and treatment of chronic hepatisis from The Japan Society of Hepatology (http://www.jsh.or.jp/medical/gudelines/index.html). The mean (and standard deviation; SD) age was 62.0 (9.4) years for the cases and 54.7 (13.5) years for the controls. The second Japanese replication sample sets for the cases (n = 142) and controls (n = 251) study were obtained from 16 hospitals. The case samples for the second replication included 142 HCC patients and the controls included 135 CHB patients and 116 asymptomatic carriers (ASC). The mean (SD) age was 61.3 (10.2) years for the cases and 56.2 (10.9) years for the controls. The Korean replication samples were collected from Yonsei University College of Medicine. The third replication set was composed of 165 HCC patients and 144 CHB patients. The mean (SD) age was 52.2 (8.9) and 37.3 (11.3) years for the cases and controls, respectively. The samples in Hong Kong were collected from the University of Hong Kong, Queen Mary Hospital. The fourth replication set was composed of 94 HCC patients and 187 CHB patients. The mean (SD) age was 58.0 (10.5) and 56.9 (8.3) years for the cases and controls, respectively. All participants provided written informed consent. This research project was approved by the Research Ethics Committees at the Institute of Medical Science and the Graduate School of Medicine, the University of Tokyo, Yonsei University College of Medicine, the University of Hong Kong, Nationa Center for Global Health and Medicine, Hokkaido University Graduate School of Medicine, Teine Keijinkai Hospital, Iwate Medical University, Saitama Medical University, Kitasato University School of Medicine, Musashino Red Cross Hospital, Kanazawa University Graduate School of Medicine, Shinshu University School of Medicine, Nagoya City University Graduate School of Medical Sciences, Kyoto Prefectural University of Medicine, National Hospital Organization Osaka National Hospital, Kawasaki Medical College, Tottori University, Ehime University Graduate School of Medicine, and Kurume University School of Medicine.

SNP Genotyping

For the first replication samples, we genotyped rs17401966 using PCR-based Invader assay (Third Wave Technologies, Madison, WI) [15], and for the second, third and fourth replication samples, we used TaqMan genotyping assay (Applied Biosystems, Carlsbad, CA). In the TaqMan SNP

genotyping assay, PCR amplification was performed in a 5-μl reaction mixture containing 1 μl of genomic DNA, 2.5 μl of KAPA PROBE FAST qPCR Master Mix (Kapa Biosystems, Woburn, MA), and 40 x TaqMan SNP Genotyping Assay probe (ABI) for this SNP. QPCR thermal cycling was performed as follows: 95°C for 3 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The SNP call rate of each replication panel was 100 %, 100 %, 99.7 % and 99.6 %.

Statistical analysis

We performed Hardy-Weinberg equilibrium test for the case and control samples in each replication study. Fisher's exact test was applied to two-by-two contingency tables for three different genetic models; allele frequency, dominant and recessive model. Odds ratios and confidence intervals were calculated using the major alleles as references. Meta-analysis was conducted using the Mantel-Haenszel method. Heterogeneity among studies was examined by using the Breslow-Day test. Genotype-phenotype association for the SNP rs17401966 was assessed using logistic regression analysis adjusted for age and gender in plink 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml).

Additional file

Additional file 1: Table S1. Samples used in this study.

Abbreviations

HB: Hepatitis b; HBV: Hepatitis b virus; HCC: Hepatocellular carcinoma; CHB: Chronic hepatitis b; HCV: Hepatitis c virus; GWAS: Genome-wide association study; ASC: Asymptomatic carrier.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

This study was supported by a grant-in-aid from the Ministry of Health, Labour, and Welfare of Japan (H23-kanen-005), and Japan Science and Technology Agency (09038024). We thank Dr. Minae Kawashima to giving us technical advices.

Author details

[1]Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. [2]The Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Japan. [3]Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [4]Department of Internal Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan. [5]Department of Internal Medicine, Teine Keijinkai Hospital, Sapporo, Japan. [6]First Department of Internal Medicine, Iwate Medical University, Iwate, Japan. [7]Division of Gastroenterology and Hepatology, Internal Medicine, Saitama Medical University, Saitama, Japan. [8]Department of Gastroenterology, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan. [9]Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan. [10]Department of Gastroenterology, Kanazawa University Graduate School of Medicine, Kanazawa, Japan. [11]Department of Medicine, Shinshu University School of Medicine, Matsumoto, Japan. [12]Department of Clinical Molecular Informative Medicine, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan. [13]Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, Japan. [14]National Hospital Organization Osaka National Hospital, Osaka, Japan. [15]Division of Hepatology and Pancreatology, Kawasaki Medical College, Kurashiki, Japan. [16]Second department of Internal Medicine, Faculty of Medicine, Tottori University, Yonago, Japan. [17]Department of Gastroenterology and Metabology, Ehime University Graduate School of Medicine, Ehime, Japan. [18]Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Fukuoka, Japan. [19]Department of International Medicine, Yonsei University College of Medicine, Seoul, Korea. [20]Department of Medicine, the University of Hong Kong, Queen Mary Hospital, Hong Kong, China.

Author contributions

Study design and discussion: H.S., N.N., Y.T., Ko.M., M.M., K.T.; sample collection: Y.T., Ko.M., Y.N., S.H.A., K.H.H., J.Y.P., M.F.Y., S.H., J.H.K., K.A., S.M., M.W., M.Ku., Y.A., N. I., M.H., S.K., E.T., Ke.M., Y.I., E.M., M.Ko., K.H., Y.Mu., Y.H., T.I., K.I., M.S., M.M.; genotyping: H.S., Y.M., M.Y., H.M.; statistical analysis: H.S.; manuscript writing: H.S., N.N., Y.T., M.M., K.T. All authors read and approved the final manuscript.

Received: 2 March 2012 Accepted: 19 June 2012
Published: 19 June 2012

References

1. Parkin DM, Bray F, Ferlay J, Pisani P: **Global cancer statistics, 2002.** *CA: a cancer journal for clinicians* 2005, **55**(2):74–108.
2. Parkin DM: **Global cancer statistics in the year 2000.** *The lancet oncology* 2001, **2**(9):533–543.
3. Marrero CR, Marrero JA: **Viral hepatitis and hepatocellular carcinoma.** *Archives of medical research* 2007, **38**(6):612–620.
4. Kamatani Y, Wattanapokayakit S, Ochi H, Kawaguchi T, Takahashi A, Hosono N, Kubo M, Tsunoda T, Kamatani N, Kumada H, *et al*: **A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians.** *Nature genetics* 2009, **41**(5):591–595.
5. Zhang H, Zhai Y, Hu Z, Wu C, Qian J, Jia W, Ma F, Huang W, Yu L, Yue W, *et al*: **Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers.** *Nature genetics* 2010, **42**(9):755–758.
6. Abdulla MA, Ahmed I, Assawamakin A, Bhak J, Brahmachari SK, Calacal GC, Chaurasia A, Chen CH, Chen J, Chen YT *et al*: **Mapping human genetic diversity in Asia.** *Science (New York, NY* 2009, **326**(5959):1541–1545.
7. Chan KY, Wong CM, Kwan JS, Lee JM, Cheung KW, Yuen MF, Lai CL, Poon RT, Sham PC, Ng IO: **Genome-wide association study of hepatocellular carcinoma in Southern Chinese patients with chronic hepatitis B virus infection.** *PLoS One* 2011, **6**(12):e28798.
8. Sherman M: **Hepatocellular carcinoma: epidemiology, surveillance, and diagnosis.** *Semin Liver Dis* 2010, **30**(1):3–16.
9. Yang HI, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, Wang LY, Lu SN, You SL, Chen DS, *et al*: **Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma.** *J Natl Cancer Inst* 2008, **100**(16):1134–1143.
10. Qian GS, Ross RK, Yu MC, Yuan JM, Gao YT, Henderson BE, Wogan GN, Groopman JD: **A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China.** *Cancer Epidemiol Biomarkers Prev* 1994, **3**(1):3–10.
11. Ross RK, Yuan JM, Yu MC, Wogan GN, Qian GS, Tu JT, Groopman JD, Gao YT, Henderson BE: **Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma.** *Lancet* 1992, **339**(8799):943–946.
12. Wang LY, Hatch M, Chen CJ, Levin B, You SL, Lu SN, Wu MH, Wu WP, Wang LW, Wang Q, *et al*: **Aflatoxin exposure and risk of hepatocellular carcinoma in Taiwan.** *International journal of cancer* 1996, **67**(5):620–625.
13. Liu Y, Wu F: **Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment.** *Environmental health perspectives* 2010, **118**(6):818–824.
14. Nakamura Y: **The BioBank Japan Project.** *Clin Adv Hematol Oncol* 2007, **5**(9):696–697.
15. Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y: **A high-throughput SNP typing system for genome-wide association studies.** *Journal of human genetics* 2001, **46**(8):471–477.

doi:10.1186/1471-2350-13-47
Cite this article as: Sawai *et al.*: No association for Chinese HBV-related hepatocellular carcinoma susceptibility SNP in other East Asian populations. *BMC Medical Genetics* 2012 **13**:47.

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Superselective transarterial chemoembolization for hepatocellular carcinoma. Validation of treatment algorithm proposed by Japanese guidelines

Kenichi Takayasu[1,*,†], Shigeki Arii[2,†], Masatoshi Kudo[3,†], Takafumi Ichida[4,†], Osamu Matsui[5,†], Namiki Izumi[6,†], Yutaka Matsuyama[7,†], Michiie Sakamoto[8,†], Osamu Nakashima[9,†], Yonson Ku[10,†], Norihiro Kokudo[11,†], Masatoshi Makuuchi[12,†]

[1]*Diagnostic Radiology, National Cancer Center Hospital, Tokyo, Japan;* [2]*Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental University Graduate School of Medicine, Tokyo, Japan;* [3]*Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Sayama, Japan;* [4]*Department of Hepatology and Gastroenterology, Juntendo Shizuoka Hospital, Izunokuni, Japan;* [5]*Department of Radiology, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan;* [6]*Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Musashino, Japan;* [7]*Department of Biostatistics, School of Public Health, University of Tokyo, Tokyo, Japan;* [8]*Department of Pathology, Keio University School of Medicine, Tokyo, Japan;* [9]*Department of Clinical Laboratory Medicine, Kurume University Hospital, Kurume, Japan;* [10]*Department of Surgery, Kobe University Graduate School of Medicine, Kobe, Japan;* [11]*Hepato-Biliary-Pancreatic Surgery Division, Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan;* [12]*Surgery, Japanese Red Cross Medical Center, Tokyo, Japan*

Background & Aims: Transcatheter arterial chemoembolization with lipiodol (TACE) is widely performed in patients with hepatocellular carcinoma (HCC) unsuitable for curative treatment. It has recently been recommended for patients with 2 or 3 tumors >3 cm or ⩾4 tumors in a treatment algorithm proposed by Japanese guidelines. However, the best indication and appropriateness of the algorithm for TACE are still unclear.

Methods: In 4966 HCC patients who underwent TACE, survival was evaluated based on tumor number, size and liver function; and the adequacy of the algorithm for TACE was validated. Exclusion criteria were: vascular invasion, extrahepatic metastasis, and prior treatment. The mean follow up period was 1.6 years.

Results: The overall median and 5-year survivals were 3.3 years and 34%, respectively. Multivariate analysis revealed that Child–Pugh class, tumor number, size, alpha-fetoprotein, and des-gamma carboxy-prothrombin were independent predictors. The survival rate decreased as the tumor number ($p = 0.0001$) and size increased ($p = 0.04$ to $p = 0.0001$) in all but one subgroup in both Child–Pugh-A and -B. The stratification of these patients to four treatments in the algorithm showed potential ability to discriminate survivals of the resection and ablation (non-TACE) groups from those of the TACE group in Child–Pugh-B and partially in A.

Conclusions: TACE showed higher survival rates in patients with fewer tumor numbers, smaller tumor size, and better liver function. The treatment algorithm proposed by the Japanese guidelines might be appropriate to discriminate the survival of patients with non-TACE from TACE therapy.

© 2011 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

Cancer

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide with 626,000 new cases every year, and is the third most common cause of death from cancer [1]. The frequency of curative treatment, such as resection, local ablation, and/or liver transplantation is low (only 30%) due to advanced cancer stage and associated liver cirrhosis at the time of diagnosis [2]. Among several treatments, transcatheter arterial chemoembolization with lipiodol (TACE) is widely performed in patients with unresectable HCC at an initial and recurrent time, which accounts for 32% and 58% of all treatment modalities, respectively, in Japan [3]. Superselective TACE is indispensable to maximize the effect in targeted tumors and to minimize liver injury [4].

Recently, two treatment algorithms for HCC were proposed: the Barcelona Clinic Liver Cancer (BCLC) classification, in 2001 [5] and the Japanese guidelines, in 2005 [6]. The first one recommends TACE in patients with multi-nodular HCC in Child–Pugh A or B in the intermediate stage, while the second recommends TACE in patients with 2 or 3 tumors, >3 cm in diameter or ⩾4 tumors in liver damage A or B. In both guidelines, vascular invasion and/or extrahepatic spread are excluded. However, the survival rate of TACE-stratified to recommended treatment of the Japanese guidelines algorithm and its appropriateness have not

Keywords: Hepatocellular carcinoma (HCC); Transcatheter arterial chemoembolization (TACE); Prognostic factor; Validation of treatment algorithm; Japanese guidelines.

Received 6 August 2011; received in revised form 15 October 2011; accepted 26 October 2011; available online 13 December 2011

* Corresponding author. Address: Diagnostic Radiology, National Cancer Center Hospital, 5-1-1, Tsukiji, Chuo-Ku, Tokyo 104-0045, Japan. Tel.: +81 3 3542 2511; fax: +81 3 3542 3815.

E-mail address: ktakayas@ncc.go.jp (K. Takayasu).

† For the Liver Cancer Study Group of Japan.

been yet determined. Thus, we conducted this research with a large scale of samples.

Patients and methods

During 6 years, from January 2000 to December 2005, a total of 60,773 patients with primary liver cancer were prospectively registered bi-annually by the Liver Cancer Study Group of Japan (LCSGJ) throughout 800 medical institutions using a registration/questionnaire sheet with more than 180 questions. Among them, 53,008 patients were clinically diagnosed with HCC with multiple imaging modalities, tumor markers, and/or needle biopsy. Four thousand nine hundred sixty-six patients were selected in the current cohort study. Inclusion criteria were the following: TACE was performed in naïve patients as an initial treatment and any other therapy such as resection and local ablation was not performed during the first investigation period within at least 2 years. Exclusion criteria were: vascular invasion of the portal and hepatic veins, invasion of the biliary duct, extrahepatic spread and history of previous treatment for HCC.

HCC was diagnosed using ultrasonography (US), dynamic computed tomography (CT), magnetic resonance imaging (MRI), and/or pathologically by biopsy specimens (3.2%). Abnormal elevation of tumor markers was also referred: alpha-fetoprotein (AFP) >400 ng/ml (normal, <20) and des-gamma carboxyl prothrombin (DCP) >100 mAU/ml (normal, <40). Typical HCC was depicted as hyper-attenuation in arterial phase and hypo-attenuation or wash-out in delayed phase (around 3 min after the beginning of contrast injection) of dynamic CT and on dynamic MRI. If the tumor showed an atypical profile and was larger than 2 cm in diameter, further examination was recommended as follows: angiography, combination of CT and angiography, MRI with super-paramagnetic iron oxide, CE-US with micro-bubble (Levovist, Bayer Schering Pharma, Germany), and/or needle biopsy. If the tumor was less than 2 cm, a follow up study with US was recommended [6]. The extrahepatic metastases were routinely examined by CT, US, and chest X-ray.

The distribution of background factors of patients with TACE is shown in Table 1. The study population predominantly consisted of patients older than 60 years [n = 4205 (85%)] and among them 3369 were male patients (68%). The proportion of Child–Pugh A/B/C was 69% (n = 3229 patients), 28% (n = 1296), and 4% (n = 167), respectively. 3479 patients (73%) were positive for hepatitis C virus antibody and 449 were positive for hepatitis B virus surface antigen. The maximum tumor size was ⩽2 cm in diameter for 32% and ⩽3 cm for 56% of tumors. The mean diameter was 3.8 ± 3.5 (standard deviation, SD) cm. The tumor number was one in 2252 patients (46%), two in 1003, three in 565, and more than four in 1092. 1868 patients (40%) had a normal AFP value and 900 had more than 401 ng/ml. 2128 patients (52%) had a DCP value ⩽100 mAU/ml.

According to the TNM stage revised by the LCSGJ in 2000 [7], 836 patients were in stage I, 2070 (43%) in stage II, and 1887 in stage III. The embolization area was less than one segment in 1589 patients (33%), equal to or more than one segment to less than one lobe in 2134 (44%), and the whole liver in only 247 patients (5%). Hypervascular HCC accounted for 98% (n = 4787 patients) and non-hypervascular HCC for 2% (n = 100). Mean bilirubin value was 1.1 ± 0.9 mg/dl (SD). Performance status (PS) according to Eastern Cooperative Oncology Group scale was PS0 in 1485 (80%) patients, PS1 in 298, PS2 in 48, PS3 in 23, and PS4 in 2 out of 1856 patients, namely 99% of patients, which were available during the last two years (January, 2004 to December, 2005) of the present study, were in PS0–2.

In most patients, the catheter tip was advanced at the nearest site of the feeding artery as possible. The emulsion of the anticancer agent and lipiodol followed by gelatin sponge particles was injected under X-ray monitoring. The dose of emulsion and particles of embolic materials was determined mainly based on the tumor size and extension. The anticancer agent used was epirubicin hydrochloride in 1490 patients (74%), doxorubicin hydrochloride in 191 patients, mitomycin C in 190 patients, and cisplatin and zinostatin stimalamer (SMANCS) in 72 patients each, for a total of 2015 patients with a mean dose of lipiodol of 4.8 ± 3.0 ml (SD), which data were available during the last two years (January, 2004 to December, 2005). The patients underwent dynamic CT or MRI with AFP and DCP measurement every three to four months, and repeated TACE was determined when local recurrence, intrahepatic metastases and/or de novo HCC was found.

To analyze the survival rate, all patients in Child–Pugh A or B were divided in four groups depending on tumor number (single, two, three, and more than four lesions). Each group was subsequently subdivided in four subgroups based on tumor size; ⩽2, 2.1 to 3.0, 3.1 to 5.0, and ⩾5.1 cm in diameter. Patients in Child–Pugh C were excluded from this analysis due to their small number (n = 167). The survival rate was calculated from the date of TACE to December 31, 2005. Patient's death was the endpoint irrespective of the cause of death. The mean follow up period was 1.6 ± 1.3 years (SD). TACE-related death was designated as death within 30 days after the initial TACE.

Treatment algorithm proposed by Japanese guidelines

The treatment algorithm proposed by Japanese guidelines [6] has six treatments determined by three factors: degree of liver damage [7], number of tumors, and tumor diameter (Fig. 1). For patients with liver damage A or B, four treatments are recommended: resection for single tumor or local ablation for single tumor ⩽2 cm and liver damage B; resection or ablation for 2 or 3 tumors ⩽3 cm; resection or TACE for 2 or 3 tumors >3 cm; TACE or hepatic arterial infusion chemotherapy for more than 4 tumors. For patients with liver damage C, liver transplantation for 1 to 3 tumors ⩽3 cm or single tumor ⩽5 cm as indicated by the Milan criteria [8], and palliative care for ⩾4 tumors are recommended. In the present study, Child–Pugh class was adopted instead of degree of liver damage because the former is widely used to evaluate liver function, especially for candidates to TACE.

The executing rate of TACE was calculated with the following formula: number of patients stratified to TACE in treatment algorithm divided by a total number of patients who actually received TACE × 100 (%). The adequacy of treatment algorithm for TACE was validated when the survivals of patients stratified to TACE group (for 2 or 3 lesions >3 cm or more than 4 lesions) and those of patients stratified to non-TACE group (such as resection and ablation for single lesion or 2 or 3 lesions ⩽3 cm) could be discriminated.

Statistical analysis

The survival rate was obtained by the Kaplan–Meier method and compared by the log-rank test in Tables 1, 2A and B, and 3. The multivariate analysis was performed with the Cox's proportional hazard model. All variables, except for one of the embolization area of the liver due to the factor obtained following TACE therapy, with p value less than 0.05 on univariate analysis, were subjected to multivariate analysis. All significance tests were two-tailed, and p value less than 0.05 was considered statistically significant. All statistical analyses were carried out with the Statistical Analysis System (SAS) version 8.02 (SAS Inc., Cary, NC).

Results

Survival rates

For overall survival of the 4966 patients who underwent TACE, the median, and 1-, 2-, 3-, 4- and 5-year survival rates were 3.3 years (40 months) and 87%, 70%, 55%, 42%, and 34%, respectively (Fig. 2). The 3- and 5-year survival in Child–Pugh A, B, and C was 61% and 40%; 43% and 22%; 23% and 0%, respectively (Table 1).

Patient characteristics analyzed by univariate and multivariate analyses

The univariate analysis revealed that there was a significant difference between the following seven variables ($p = 0.0001$); Child–Pugh class, maximum tumor size, number of lesions, AFP, DCP, TNM stage, and extent of embolization area (Table 1).

The multivariate analysis showed that the following five variables were independent predictors in trial 1; Child–Pugh class, tumor size, number of lesions, AFP, and DCP (Supplementary Table 1). In trial 2, where tumor size and number of lesions in trial 1 were replaced by TNM stage, four variables were independent predictors: Child–Pugh class, TNM stage, AFP, and DCP.

Survival rates of patients stratified to four groups divided by lesion number and to four subgroups subdivided by lesion size

In Child–Pugh A patients (n = 3194), the overall median and 3-year survival rate in four groups divided by tumor number: single, two, three, and more than 4 lesions, were 5.4 years and 73%, 3.8 years and 59%, 3.1 years and 52%, and 2.8 years and

Table 1. Distribution of background factors and results of the univariate analysis in 4966 patients with hepatocellular carcinoma who underwent transcatheter arterial chemoembolization with lipiodol.

Background factors	No. of patients	Proportion (%)	Survivals (%)				*p*	Hazard ratio (95% CI)
			1-yr	3-yr	4-yr	5-yr		
Age, yr							0.88	
<60	756	15	85	56	43	39		Ref.
≥60	4205	85	87	55	42	33		1.01 (0.88, 1.16)
Gender							0.40	
M	3369	68	86	54	42	35		1.05 (0.93, 1.18)
F	1597	32	89	56	42	33		Ref.
Child-Pugh classification							0.0001	
A	3229	69	90	61	49	40		Ref.
B	1296	28	82	43	27	22		1.81 (1.62, 2.04)
C	167	4	69	23	12	-		3.05 (2.44, 3.81)
HBV and HCV							0.50	
HCV Ab positive	3479	73	87	55	41	34		1.01 (0.87, 1.18)
HBs Ag positive	449	9	84	53	37	35		1.11 (0.90, 1.38)
Both positive	89	2	89	58	54	43		0.83 (0.56, 1.25)
Both negative	768	16	86	56	44	32		Ref.
Maximum tumor size (cm)							0.0001	
≤2	1549	32	93	65	50	42		Ref.
2.1-3	1178	24	89	53	42	35		1.38 (1.19, 1.60)
3.1-5	1291	27	85	52	37	29		1.62 (1.41, 1.87)
≥5.1	811	17	77	44	34	23		2.19 (1.88, 2.56)
No. of lesions							0.0001	
1	2252	46	91	66	53	45		Ref.
2	1003	20	88	55	42	34		1.35 (1.17, 1.56)
3	565	12	86	45	27	20		1.77 (1.50, 2.08)
≥4	1092	22	79	39	30	20		2.18 (1.91, 2.48)
Alpha-fetoprotein (ng/ml)							0.0001	
≤20	1868	40	92	64	50	44		Ref.
21-200	1613	34	89	55	40	30		1.38 (1.21, 1.57)
201-400	311	7	82	45	33	29		1.73 (1.40, 2.14)
401-1000	309	7	81	43	32	21		2.07 (1.68, 2.55)
≥1001	591	13	72	38	32	23		2.49 (2.13, 2.92)
Des-gamma carboxy-prothrombin (mAU/ml)							0.0001	
≤100	2128	52	92	65	52	40		Ref.
101-299	599	15	88	52	41	32		1.50 (1.26, 1.78)
300-499	245	6	84	49	27	24		1.93 (1.54, 2.42)
500-999	294	7	82	50	33	20		1.89 (1.51, 2.36)
≥1000	794	20	76	38	26	18		2.52 (2.18, 2.91)
TNM stage							0.0001	
I (T1N0M0)	836	17	93	72	59	51		Ref.
II (T2N0M0)	2070	43	90	60	46	37		1.51 (1.27, 1.80)
III (T3N0M0)	1887	39	81	42	30	22		2.60 (2.19, 3.09)
Extent of embolization							0.0001	
<one segment	1589	33	90	64	51	44		Ref.
1 seg. ≤ to <1 lobe	2134	44	87	55	41	32		1.33 (1.17, 1.51)
1 lobe ≤ to <whole liver	873	18	85	47	32	23		1.67 (1.43, 1.94)
Whole liver	247	5	74	37	29	-		2.27 (1.85, 2.80)

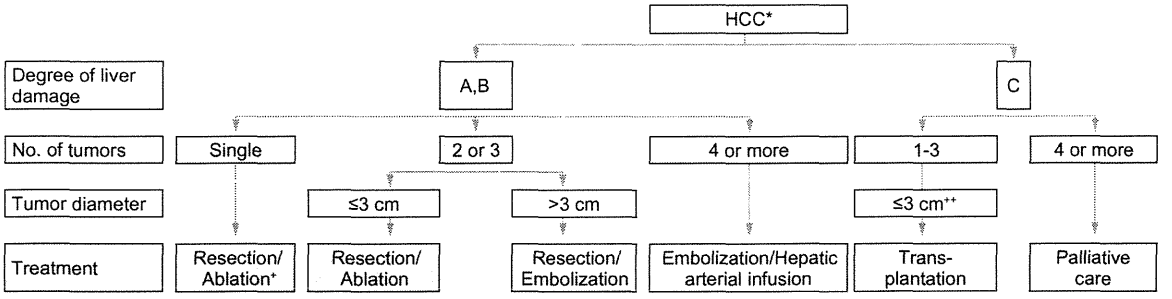

Fig. 1. Treatment algorithm for HCC proposed by Japanese guidelines. (+) shows local ablation for single lesion ≤2 cm in patients with liver damage B. (++) means liver transplantation for no more than 3 lesions ≤3 cm or single lesion ≤5 cm. The asterisk shows that for patients with vascular invasion and liver damage A, hepatectomy, TACE or hepatic arterial infusion chemotherapy may be recommended, while chemotherapy is an option for patients with extrahepatic metastasis.

46%, respectively ($p = 0.0001$, Table 2A). The survival rate of four subgroups subdivided by tumor size from ≤2 cm to ≥5.1 cm decreased as the lesion size increased in all ($p = 0.04$ to $p = 0.0001$) but one group with 3 lesions ($p = 0.07$). The highest 3-year survival was 80% in patients with single lesion ≤2 cm, and the lowest 3-year survival was 30% in patients with more than 4 lesions ≥5.1 cm.

In Child–Pugh B (n = 1284), the overall median and 3-year survival rate of four groups were 3.1 years and 53%, 2.8 years and 49%, 2.0 years and 24%, and 1.9 years and 22%, respectively ($p = 0.0001$, Table 2B). The survival rate of four subgroups divided by tumor size in each group decreased as the lesion size increased in all ($p = 0.01$ to $p = 0.0004$) but one group with single lesion ($p = 0.49$). The highest 3-year survival was 65%, found in patients with 2 lesions ≤2 cm, and the lowest was 0% in patients with three lesions ≥5.1 cm.

Validation of the treatment algorithm proposed by the Japanese guidelines

Of 3168 patients with TACE in Child–Pugh A, 1475 were stratified to resection or ablation therapy for single lesion in the treatment algorithm (Fig. 1), 506 to resection or ablation for 2 or 3 lesions ≤3 cm, 463 to resection or TACE for 2 or 3 lesions >3 cm, and 724 to TACE or hepatic arterial infusion chemotherapy for ≥4 lesions (Table 3). The median and 3-year survival rates of the corresponding four treatments were 5.4 years and 73%, 3.5 years and 59%, 3.4 years and 55%, and 2.8 years and 46%, respectively, with a significant difference ($p = 0.0001$). The comparisons of the survival curves between two treatment groups showed a significant difference in all ($p = 0.013$ to $p = 0.0001$) but one comparison between treatments for 2 or 3 lesions ≤3 cm and for 2 or 3 lesions >3 cm ($p = 0.06$) (Fig. 3). Namely, survival discrimination was feasible between one of two TACE treatments for >4 lesions and non-TACE therapies such as resection or ablation.

Similarly, 1274 patients with Child–Pugh B were stratified to four treatment categories (Table 3). The median and 3-year survivals of these treatments from single to ≥4 lesions were 3.1 years and 53%, 2.8 years and 49%, 1.7 years and 30%, and 1.9 years and 22%, respectively, with a significant difference ($p = 0.0001$). The comparisons of survival curves between two treatment groups showed a significant difference in all ($p = 0.0001$) but two comparisons; single lesion *vs.* 2 or 3 lesions ≤3 cm ($p = 0.79$) and 2 or 3 lesions >3 cm *vs.* ≥4 lesions ($p = 0.84$) (Fig. 4). Namely, survival discrimination was feasible between two TACE therapy groups, i.e., 2 or 3 lesions >3 cm and ≥4 lesions and two non-TACE groups.

The executing rate of TACE was 37% in both Child–Pugh A (1187/3168 patients) and B (467/1274).

TACE-related mortality rate

After the initial TACE, treatment-related death occurred in 19 (0.38%) out of 4966 patients. The breakdown of the cause of death was cancer in 5 patients (26%), hepatic failure in 3 (16%), rupture of esophago-gastric varices in one patient, intra-peritoneal rupture of HCC in another patient, and other causes in 9 patients. Ten patients were in Child–Pugh A, 8 were in class B and one was in class C.

Discussion

The present study demonstrates that the overall median and 3-, and 5-year survival rates of TACE were 3.3 years (40 months), 55%, and 34%, respectively, and were better than those previously reported by the LCSGJ (34 months, 47%, and 26% [9]), mainly due to exclusion criteria of vascular invasion in the current study. The multivariate analysis revealed that five variables were independent predictors in trial 1: Child–Pugh class, tumor size, tumor number, AFP, and DCP; and four variables in trial 2, where tumor size and tumor number were replaced by TNM stage. These results are similar to those of a previous study [9] other than Child–Pugh class instead of degree of liver damage and DCP value were newly adopted.

There was an inverse correlation between tumor number and overall survival of patients with TACE therapy ($p = 0.0001$) in both Child–Pugh A and B (Tables 2A and B) as well as between tumor diameter and survival in all but one group, each in Child–Pugh A and B. Namely, the fewer the tumor number and the smaller the tumor size, the better the survival rates. The best 3-year survival (80%) was found in patients with a single HCC ≤2 cm in Child–Pugh A, and the worst 3-year survival (0%) in patients with three lesions ≥5.1 cm in class B. However, in clinical practice, the best survivor with TACE is not recommended to TACE but to resection or local ablation due to relatively higher 3-year survival rates, 90% and 85%, respectively [3]. The current study has revealed a wide range of survival rates for patients with

Table 2. The overall survivals of four groups divided by tumor number and survivals of four subgroups divided by tumor size in patients who underwent TACE. (A) Child–Pugh A (n = 3194 patients). (B) Child–Pugh B (n = 1284).

A

Group/ subgroup	No. of patients	Survival (%) 1-yr	3-yr	4-yr	5-yr	Median (yr)	p	Hazard ratio (95% CI)
Single lesion								
Overall*	1475	93	73	62	52	5.4		
≤2 cm	546	97	80	73	65	-	0.0001	Ref.
2.1-3.0	353	92	71	56	36	4.5		1.88 (1.36, 2.62)
3.1-5.0	328	92	66	53	46	4.6		1.97 (1.42, 2.75)
≥5.1	219	86	66	48	-	4.2		2.38 (1.64, 3.46)
Two lesions								
Overall*	634	91	59	47	40	3.8		
≤2 cm	178	97	64	49	42	3.9	0.04	Ref.
2.1-3.0	144	91	50	39	-	2.8		1.57 (1.01, 2.44)
3.1-5.0	190	90	66	47	39	4.0		1.23 (0.80, 1.90)
≥5.1	104	84	53	45	38	4.1		1.94 (1.19, 3.15)
Three lesions								
Overall*	361	90	52	33	24	3.1		
≤2 cm	102	92	65	30	-	3.6	0.07	Ref.
2.1-3.0	82	95	51	32	-	3.1		1.16 (0.68, 1.99)
3.1-5.0	111	94	48	38	-	3.0		1.30 (0.80, 2.10)
≥5.1	58	73	35	-	-	2.2		2.02 (1.19, 3.45)
More than 4 lesions								
Overall*	724	82	46	37	25	2.8		
≤2 cm	168	92	59	54	44	4.4	0.0001	Ref.
2.1-3.0	137	83	54	51	32	4.0		1.47 (0.97, 2.25)
3.1-5.0	207	82	43	25	16	2.5		2.00 (1.38, 2.90)
≥5.1	190	74	30	18	-	1.7		2.89 (2.01, 4.17)

B

Group/ subgroup	No. of patients	Survival (%) 1-yr	3-yr	4-yr	5-yr	Median (yr)	p	Hazard ratio (95% CI)
Single lesion								
Overall*	568	87	53	34	30	3.1		
≤2 cm	213	89	56	33	24	3.3	0.49	Ref.
2.1-3.0	169	88	50	44	39	2.9		1.00 (0.69, 1.44)
3.1-5.0	132	87	54	20	-	3.1		1.24 (0.83, 1.86)
≥5.1	47	78	49	-	-	2.5		1.40 (0.80, 2.47)
Two lesions								
Overall*	276	83	49	34	-	2.8		
≤2 cm	86	93	65	48	-	4.0	0.0004	Ref.
2.1-3.0	70	93	43	21	-	2.3		1.86 (1.04, 3.34)
3.1-5.0	82	69	41	27	-	2.1		2.49 (1.47, 4.21)
≥5.1	31	58	-	-	-	1.5		3.66 (1.83, 7.32)
Three lesions								
Overall*	150	77	24	14	-	2.0		
≤2 cm	40	89	28	19	-	2.0	0.005	Ref.
2.1-3.0	43	76	30	-	-	2.3		1.09 (0.50, 2.35)
3.1-5.0	41	73	34	17	-	1.8		1.31 (0.65, 2.64)
≥5.1	23	62	-	-	-	1.4		3.16 (1.49, 6.72)
More than 4 lesions								
Overall*	290	72	22	10	-	1.9		
≤2 cm	57	90	32	24	-	2.0	0.01	Ref.
2.1-3.0	68	75	17	8	-	2.1		1.53 (0.85, 2.76)
3.1-5.0	89	73	25	-	-	2.0		1.54 (0.88, 2.69)
≥5.1	65	54	-	-	-	1.2		2.55 (1.43, 4.56)

*A significant difference was demonstrated in overall survival among four groups ($p = 0.0001$).

TACE, mainly because of the heterogeneity of the population, therefore it would be helpful for candidates to determine chemoembolization as tailor-made treatment of choice; this is particularly suitable for patients averse to curative therapy and with severely associated diseases, or elderly patients.

The executing rate of patients who actually had undergone TACE and were stratified to TACE in the treatment algorithm was 37% in both Child–Pugh A and B. Namely, the remaining 63% of patients satisfied the criteria of resection or local ablation (non-TACE therapy), which could suggest the possible increase of survival in these patients, if they underwent resection or local ablation. The reason for the lower executing rate might be the less publicity in which the guideline was published one year after the completion of this 6-year study.

The discrimination of patients' survival was feasible in this treatment algorithm between TACE and non-TACE therapies in Child–Pugh B and in part in class A. Further studies are needed to validate the suitability of these guidelines using patients who underwent resection or local ablation and are stratified to four treatments like in the TACE study.

To our knowledge, the present study is the first report to clarify the median and 3- and 4-year survivals of patients treated by TACE and stratified in the four treatments recommended by the Japanese guidelines in Child–Pugh A and B, separately. Interestingly, Llovet *et al.* [10] stated that chemoembolization improved median survival up to 19–20 months in intermediate stage of BCLC classification, which is similar to our results; 1.7 years (20 months) in patients with TACE for 2 or 3 lesions >3 cm and

Table 3. Survival rates of patients treated with TACE stratified to four treatment categories recommended by Japanese guidelines in Child–Pugh A and B.

Criteria of treatment	No. of patients	Survival (%)				Median	*p*	Hazard ratio (95% CI)
		1-yr	3-yr	4-yr	5-yr	(yr)		
Child-Pugh A	3168							
Single lesion	1475	93	73	62	52	5.4	0.0001	Ref.
2-3 lesions, ≤3 cm	506	94	59	40	36	3.5		1.45 (1.18, 1.78)
2-3 lesions, >3 cm	463	87	55	44	31	3.4		1.82 (1.48, 2.25)
≥4 lesions	724	82	46	37	25	2.8		2.39 (2.01, 2.84)
Child-Pugh B	1274							
Single lesion	568	87	53	34	30	3.1	0.0001	Ref.
2-3 lesions, ≤3 cm	239	90	49	33	-	2.8		1.04 (0.79, 1.36)
2-3 lesions, >3 cm	177	68	30	19	-	1.7		2.11 (1.62, 2.75)
≥4 lesions	290	72	22	10	-	1.9		2.17 (1.72, 2.74)

Fig. 2. Overall survival rate of 4966 HCC patients who underwent TACE.

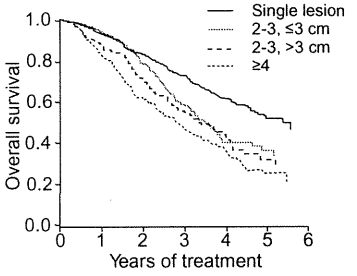

Fig. 3. The survival curves of 3168 patients in Child–Pugh A stratified to four treatment groups according to Japanese guidelines. Overall, there was a significant difference ($p = 0.0001$). There was also a significant difference between two treatment groups except for one; 2 or 3 lesions ≤3 cm *vs.* 2 or 3 lesions >3 cm ($p = 0.06$).

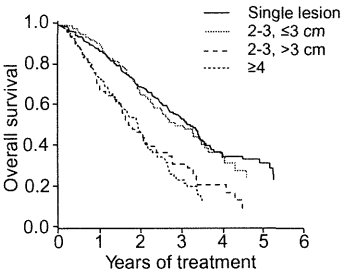

Fig. 4. The survival curves of 1274 patients in Child–Pugh B stratified to four treatment groups. Overall, a significant difference was seen ($p = 0.0001$). A significant difference was observed between two groups except for two; single lesion *vs.* 2 or 3 lesions ≤3 cm, and 2 or 3 lesions >3 cm *vs.* ≥4 lesions.

1.9 years (23 months) in those for ≥4 lesions in Child–Pugh B (Table 3). If the criteria for TACE are similar in the intermediate stage of the BCLC staging system [5] and in the treatment algorithm of the Japanese guidelines: equal to or more than 4 lesions and/or 2 or 3 lesions >3 cm [11], the current data will be useful to compare the survival outcomes of TACE in the East and West. The survival rates of our study will be also used as reference data when clinical trials of TACE with or without anti-angiogenic drugs are newly designed [12,13].

The treatment-related mortality rate was 0.38%, which was slightly improved compared to that of our previous study of 0.5% [9], and much better than that of 2.4% reported by a systematic review [14]. The improvement is mainly attributable to the exclusion criteria of vascular or biliary duct invasion and the decreased proportion of Child–Pugh C patients, from 10% [9] to 4%.

As a limitation of this study, the session numbers of TACE per patient and dosage of anticancer agent used at initial TACE were not available due to lack of inclusion in the questionnaire sheet. Our patients received different TACE protocols for anticancer agent. Given that the large majority of patients were treated with epirubicin or doxorubicin, it could be worth limiting the analysis to these patient cohorts.

In conclusion, the overall median and 3- and 5-year survival of TACE were 3.3 years, 55% and 34% in 4966 HCC patients without vascular invasion and extrahepatic spread. The tumor number, size, liver function, AFP, and DCP were independent predictors. These results will be helpful for physicians to select chemoembolization as optimal therapy for their patients, especially when curative treatment is contraindicated due to severely associated disease and/or aging. The treatment algorithm of the Japanese guidelines might be appropriate to discriminate patient survival

with non-TACE from TACE therapy in Child–Pugh B and in part in A. The survival rates of patients stratified to TACE in these guidelines will be useful for comparing the outcome of TACE in the East and West, and for designing new clinical trials for TACE with and without a novel molecular targeted agent as reference data.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2011.10.021.

References

[1] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
[2] Bruix J, Llovet JM. Prognostic prediction and treatment strategy in hepatocellular carcinoma. Hepatology 2002;35:519–524.
[3] Ikai I, Kudo M, Arii S, Omata M, Kojiro M, Sakamoto M, et al. Report of the 18th follow up survey of primary liver cancer in Japan. Hepatol Res 2010;40:1043–1059.
[4] Matsui O, Kadoya M, Yoshikawa J, Gabata T, Arai K, Demachi H, et al. Small hepatocellular carcinoma: treatment with subsegmental transcatheter arterial embolization. Radiology 1993;188:79–83.
[5] Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001;35:421–430.
[6] Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, et al. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res 2008;38:37–51.
[7] The Liver Cancer Study Group of Japan. The general rules for the clinical and pathological study of primary liver cancer. 2nd English ed. Tokyo, Japan: Kanehara & Co., Ltd.; 2003.
[8] Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med 1996;334:693–699.
[9] Takayasu K, Arii S, Ikai I, Omata M, Okita K, Ichida T, et al. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. Gastroenterology 2006;131:461–469.
[10] Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008;100:698–711.
[11] Takayama T. Hepatocellular carcinoma. In: Clavien PA, editor. Malignant liver tumors: current and emerging therapies. London: Wiley-Blackwell; 2010. p. 317–323.
[12] Sergio A, Cristofori C, Cardin R, Pivetta G, Ragazzi R, Baldan A, et al. Transcatheter arterial chemoembolization (TACE) in hepatocellular carcinoma (HCC): the role of angiogenesis and invasiveness. Am J Gastroenterol 2008;103:914–921.
[13] Lencioni R, Zou J, Leberre M, Meinhardt G, Voliotis D, Bruix J, et al. Sorafenib (SOR) or placebo (PL) in combination with transarterial chemoembolization (TACE) for intermediate-stage hepatocellular carcinoma (SPACE). ASCO Meeting Abstracts 2010;28:TPS178.
[14] Marelli L, Stigliano R, Triantos C, Senzolo M, Cholongitas E, Davies N, et al. Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. Cardiovasc Intervent Radiol 2007;30:6–25.

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

LecT-Hepa, a Glyco-Marker Derived from Multiple Lectins, as a Predictor of Liver Fibrosis in Chronic Hepatitis C Patients

Kiyoaki Ito,[1] Atsushi Kuno,[2] Yuzuru Ikehara,[2] Masaya Sugiyama,[1] Hiroaki Saito,[1] Yoshihiko Aoki,[1] Teppei Matsui,[1] Masatoshi Imamura,[1] Masaaki Korenaga,[1] Kazumoto Murata,[1] Naohiko Masaki,[1] Yasuhito Tanaka,[3] Shuhei Hige,[4] Namiki Izumi,[5] Masayuki Kurosaki,[5] Shuhei Nishiguchi,[6] Michiie Sakamoto,[7] Masayoshi Kage,[8] Hisashi Narimatsu,[2] and Masashi Mizokami[1]

Assessment of liver fibrosis in patients with chronic hepatitis C (CHC) is critical for predicting disease progression and determining future antiviral therapy. LecT-Hepa, a new glyco-marker derived from fibrosis-related glyco-alteration of serum alpha 1-acid glycoprotein, was used to differentiate cirrhosis from chronic hepatitis in a single-center study. Herein, we aimed to validate this new glyco-marker for estimating liver fibrosis in a multicenter study. Overall, 183 CHC patients were recruited from 5 liver centers. The parameters *Aspergillus oryzae* lectin (AOL) / *Dature stramonium* lectin (DSA) and *Maackia amurensis* lectin (MAL)/DSA were measured using a bedside clinical chemistry analyzer in order to calculate LecT-Hepa levels. The data were compared with those of seven other noninvasive biochemical markers and tests (hyaluronic acid, tissue inhibitor of metalloproteases-1, platelet count, aspartate aminotransferase-to-platelet ratio index [APRI], Forns index, Fib-4 index, and Zeng's score) for assessing liver fibrosis using the receiver-operating characteristic curve. LecT-Hepa correlated well with the fibrosis stage as determined by liver biopsy. The area under the curve (AUC), sensitivity, and specificity of LecT-Hepa were 0.802, 59.6%, and 89.9%, respectively, for significant fibrosis; 0.882, 83.3%, and 80.0%, respectively, for severe fibrosis; and 0.929, 84.6%, and 88.5%, respectively, for cirrhosis. AUC scores of LecT-Hepa at each fibrosis stage were greater than those of the seven aforementioned noninvasive tests and markers. *Conclusion*: The efficacy of LecT-Hepa, a glyco-marker developed using glycoproteomics, for estimating liver fibrosis was demonstrated in a multicenter study. LecT-Hepa given by a combination of the two glyco-parameters is a reliable method for determining the fibrosis stage and is a potential substitute for liver biopsy. (HEPATOLOGY 2012;56:1448-1456)

Accurate staging of hepatic fibrosis in patients with chronic hepatitis C (CHC) is most important for predicting disease progression and determining the need for initiating antiviral therapy, such as interferon (IFN) therapy.[1,2] Liver biopsy has been considered the gold standard for fibrosis staging for many years.[3] However, liver biopsy is invasive and painful,[4,5] with rare but potentially life-threatening complications.[6] In addition, this method may suffer from sampling errors since only 1/50,000 of the organ is examined.[7] Furthermore, inter- and intraobserver discrepancies reaching levels of 10% to 20% have been

Abbreviations: α2-MG, α2-macroglobulin; AFP, alpha-fetoprotein; AGP, alpha-1 acid glycoprotein; ALT, alanine aminotransferase; AOL, Aspergillus oryzae *lectin; CHC, chronic hepatitis C; DSA,* Datura stramonium *lectin; GGT, gamma-glutamyltransferase; HA, hyaluronic acid; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; MAL,* Maackia amurensis *lectin; TIMP1, tissue inhibitors of metalloproteinases 1.*

From the [1]Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Japan; [2]Research Center for Medical Glycoscience, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan; [3]Department of Virology & Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan; [4]Department of Internal Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [5]Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan; [6]Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan; [7]Pathology, School of Medicine, Keio University, Japan; [8]Department of Pathology, Kurume University School of Medicine, Japan.

Received February 6, 2012; accepted April 22, 2012.

Supported by a grant (22-108) from the National Center for Global Health and Medicine in Japan and a grant from New Energy and Industrial Technology Development Organization of Japan.

reported using this method, leading to misdiagnosis of cirrhosis.[8] Therefore, finding a noninvasive method for diagnosing liver fibrosis is an emerging issue in the care of patients with CHC.

Several methods have been studied for the noninvasive diagnosis of hepatic fibrosis or cirrhosis, including clinical[9] or blood markers,[10,11] and signal analysis (ultrasonography, magnetic resonance imaging, and elastography).[12,13] Although each method can play a substantial role in the diagnosis of cirrhosis, it is evident that the best way of monitoring hepatitis progression employs an accurate serological method for the quantitative evaluation of fibrosis. We developed a new glyco-marker using multiple lectins that performed well in estimating liver fibrosis in a single-center study.[14,15]

Recent progress in glycoproteomics has had a great influence on work toward ideal, disease-specific biomarkers for a number of conditions. Glycoproteins that exhibit disease-associated glyco-alteration and are present in serum or other fluids have the potential to act as biomarkers for the diagnosis of a target disease,[16] because the features of glycosylation depend on the extent of cell differentiation and the stage of the cell. Detecting hepatic disease-associated glyco-markers for clinical applications has been a continuous challenge since the early 1990s, because increased fucosylation on complex-type *N*-glycans has been frequently detected in glycoproteins from patients with hepatocellular carcinoma (HCC) and cirrhosis.[17,18] Of all the alpha-fetoprotein (AFP) glycoforms, more than 30% have been found to react to a fucose-binding lectin, *Lens culinaris* agglutinin. This fraction, designated AFP-L3, was approved by the U.S. Food and Drug Administration (FDA) in 2005 for the diagnosis and prognosis of HCC.[19] We have found that two fibrosis-indicator lectins (*Aspergillus oryzae* lectin [AOL] and *Maackia amurensis* lectin [MAL]) together with an internal, standard lectin (*Datura stramonium* lectin [DSA]) on an alpha 1-acid glycoprotein (AGP) could, using lectin microarray, clearly distinguish between cirrhosis and chronic hepatitis patients.[14] We have further simplified this quantitative method so that it could be performed using bedside, clinical chemistry analyzers.[15]

The aim of the current study was to evaluate this new glyco-marker (LecT-Hepa) using multiple lectins and bedside clinical chemistry analyzers for use in the assessment of liver fibrosis. In this multicenter study we compared the method's efficiency in estimating liver fibrosis with other noninvasive fibrosis markers and tests.

Materials and Methods

Study Population. This study included 183 consecutive adult patients with CHC who had undergone percutaneous liver biopsy at one of the following institutions: Hokkaido University Hospital, Musashino Red Cross Hospital, National Center for Global Health and Medicine, Hyogo College of Medicine Hospital, or Nagoya City University Hospital in Japan. A diagnosis of CHC was defined as detectable serum anti-hepatitis C virus (HCV) antibody and HCV-RNA, found using polymerase chain reaction assays, of at least 2 points. Exclusion criteria were coinfection with hepatitis B virus or human immunodeficiency virus (HIV), and other disorders that commonly cause liver diseases. Informed consent was obtained from each patient who participated in the study. This study was conducted in accordance with the provisions of the Declaration of Helsinki and was approved by our Institutional Review Board.

Histological Staging. Ultrasonography-guided liver biopsy was performed according to a standardized protocol. Specimens were fixed, paraffin-embedded, and stained with hematoxylin-eosin and Masson's trichrome. A minimum of six portal tracts in the specimen were required for diagnosis. All liver biopsy samples were independently evaluated by two senior pathologists who were blinded to the clinical data. Liver fibrosis stages were assessed using METAVIR fibrosis (F) staging.[20] Significant fibrosis was defined as METAVIR F $\geq$2, severe fibrosis as METAVIR F $\geq$3, and cirrhosis as METAVIR F4. Two patients were excluded from the study because of inadequate histological samples.

Clinical and Biological Data. The age and sex of the patients were recorded. Serum samples were collected immediately before or no more than 2 months

Address reprint requests to: Masashi Mizokami, M.D., Ph.D., Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, 1-7-1, Konodai, Ichikawa 272-8516, Japan. E-mail: mmizokami@hospk.ncgm.go.jp; fax: +81-(0)47-375-4766.

Copyright © 2012 by the American Association for the Study of Liver Diseases.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.25815

Potential conflict of interest: Nothing to report.

after liver biopsy and were stored at −80°C until analysis. The concentrations of the following variables were obtained by analyzing the serum samples: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total bilirubin, albumin, cholinesterase, total cholesterol, platelet count (platelets), prothrombin time, haptoglobin, hyaluronic acid (HA), α2-macroglobulin (α2-MG), tissue inhibitors of metalloproteinases 1 (TIMP1). The aspartate aminotransferase-to-platelet ratio index (APRI), Fib-4 index, Forns index, and Zeng's score were calculated according to published formulae appropriate to each measure.[2,7,21,22]

Rapid Lectin-Antibody Sandwich Immunoassay Using HISCL. Fibrosis-specific glyco-alteration of AGP was qualified from simultaneous measurements of the lectin-antibody sandwich immunoassays using three lectins (DSA, MAL, and AOL). In principle, the glycan part of the AGP was captured by the lectin immobilized on the magnetic beads, and the captured AGP was then quantified by an antihuman AGP mouse monoclonal antibody probe that was cross-linked to an alkaline phosphatase (ALP-αAGP). The assay manipulation was fully automated using a chemiluminescence enzyme immunoassay machine (HISCL-2000i; Sysmex, Kobe, Japan). We used the following criterion formula, named the "LecT-Hepa Test," to enhance the diagnostic accuracy by combining two glyco-parameters (AOL/DSA and MAL/DSA) as described before: $F = \mathrm{Log}_{10}[\mathrm{AOL/DSA}]*8.6\text{-}[\mathrm{MAL/DSA}]$.[15]

Statistical Analyses. Quantitative variables were expressed as the mean ± standard deviation (SD) unless otherwise specified. Categorical variables were compared using a chi-squared test or Fisher's exact test, as appropriate, and continuous variables were compared using the Mann-Whitney U test. $P < 0.05$ was considered statistically significant. A multivariate forward stepwise logistic regression analysis was performed to determine the independent predictors of the absence or presence of significant fibrosis, severe fibrosis, and cirrhosis, respectively. Pearson's correlation coefficient was used as necessary. To assess the classification efficiencies of various markers for detecting significant fibrosis, severe fibrosis, and cirrhosis,[23] and to determine area under the curve (AUC) values, receiver-operating characteristic (ROC) curve analysis was also carried out. Diagnostic accuracy was expressed as the diagnostic specificity (specificity), diagnostic sensitivity (sensitivity), positive predictive values (PPV), negative predictive values (NPV), positive likelihood ratio (LR [+]), negative likelihood ratio (LR [−]), and AUC (95% confidence interval [95% CI]). We performed statistical analyses using STATA v. 11.0 (StataCorp, College Station, TX).

Table 1. Baseline Characteristics of the 183 Patients with Chronic Hepatitis C at the Time of Liver Biopsy

Features	Total (n = 183)
Age (years)	57.6 ± 11.4
Male sex	75 (41.0)
AST (IU/L)	57.4 ± 43.9
ALT (IU/L)	62.8 ± 56.8
GGT (IU/L)	51.1 ± 62.6
Bilirubin (mg/dL)	0.7 ± 0.4
Albumin (g/L)	4.1 ± 0.4
Cholinesterase (IU/L)	283.5 ± 97.0
Cholesterol (mg/dL)	174.1 ± 35.5
Platelets (10^9/L)	163 ± 57
Prothrombin time (%)	87.2 ± 33.4
α2-MG (g/L)	356.8 ± 133.1
HA (μg/L)	205.3 ± 428.0
TIMP1 (pg/ml)	210.6 ± 87.7
AOL/DSA	6.3 ± 12.3
MAL/DSA	9.0 ± 3.1
Fibrosis stage (%):	
F0-1	89 (48.6)
F2	46 (25.1)
F3	22 (12.0)
F4	26 (14.2)

Results

Baseline Characteristics of the 183 Patients with Chronic Hepatitis C at the Time of Liver Biopsy. Patient characteristics at the time of liver biopsy are shown in Table 1. The mean age of the 183 patients was 57.6 ± 11.4 years, and 75 (41%) of them were men. F0-F1 was diagnosed in 89 cases (48.6%), F2 in 46 (25.1%), F3 in 22 (12.0%), and F4 (cirrhosis) in 26 (14.2%).

Comparison of Variables Associated with the Presence of Significant Fibrosis by Univariate and Multivariate Analysis. Variables associated with the presence of significant fibrosis were assessed by univariate and multivariate analysis (Table 2). The variables of age ($P = 0.001$), AST ($P < 0.0001$), ALT ($P < 0.0001$), GGT ($P < 0.0001$), bilirubin ($P = 0.014$), α2-MG ($P = 0.002$), HA ($P < 0.0001$), TIMP1 ($P < 0.0001$), and AOL/DSA ($P < 0.0001$) were significantly higher in the significant fibrosis group than in the not significant fibrosis group. The variables albumin ($P < 0.001$), cholinesterase ($P < 0.0001$), cholesterol ($P = 0.005$), platelets ($P < 0.0001$), prothrombin time ($P = 0.0001$), and MAL/DSA ($P < 0.0001$) were significantly lower in the significant fibrosis group than in the not significant fibrosis group. Multivariate analysis showed that platelets (odds ratio [OR]: 0.87,

Table 2. Variables Associated with the Presence of Significant Fibrosis (F2-4) and Severe Fibrosis (F3-4) by Univariate and Multivariate Analysis

Features	No Significant Fibrosis (n = 89)	Significant Fibrosis (n = 94)	*P* Value (Univariate)	Odds Ratio (95% CI) (Multivariate)	No Severe Fibrosis (n = 135)	Severe Fibrosis (n = 48)	*P* Value	Odds Ratio (95% CI) (Multivariate)
Age (years)	54.7 ± 11.8	60.5 ± 10.4	0.001		55.8 ± 11.9	62.9 ± 7.8	0.001	1.15 (1.02-1.31)
Male sex (%)	30 (33.7)	45 (47.9)	0.051		52 (38.5)	23 (47.9)	0.255	
AST (IU/L)	45.7 ± 41.6	68.3 ± 43.5	<0.0001		49.7 ± 40.1	79.1 ± 47.4	<0.0001	
ALT (IU/L)	51.0 ± 56.6	74.0 ± 54.9	<0.0001		55.9 ± 54.9	82.5 ± 57.9	<0.0001	
GGT (IU/L)	40.6 ± 61.7	62.1 ± 63.1	<0.0001		45.5 ± 67.1	65.8 ± 46.7	<0.0001	
Bilirubin (mg/dL)	0.6 ± 0.3	0.7 ± 0.4	0.014		0.6 ± 0.3	0.8 ± 0.4	0.005	
Albumin (g/L)	4.2 ± 0.3	4.0 ± 0.5	<0.001		4.2 ± 0.3	3.8 ± 0.5	<0.0001	
Cholinesterase (IU/L)	329.2 ± 76.0	247.2 ± 96.9	<0.0001		312.4 ± 84.4	217 ± 91.9	<0.0001	
Cholesterol (mg/dL)	181.0 ± 31.5	167.5 ± 36.2	0.005		178.1 ± 34.1	162.4 ± 33.5	0.016	
Platelets (10^9/L)	186 ± 53	142 ± 52	<0.0001	0.87 (0.77-0.99)	180 ± 52	119 ± 46	<0.0001	0.74 (0.58-0.94)
Prothrombin time (%)	94.7 ± 33.4	80.1 ± 32.1	0.0001		89.5 ± 36.2	80.8 ± 23.2	<0.001	
α2-MG (g/L)	326 ± 117.7	389.2 ± 141.1	0.002		331.1 ± 122.5	423.9 ± 137.5	<0.0001	
HA (μg/L)	85.6 ± 154.3	318.7 ± 556.1	<0.0001	1.01 (1.01-1.02)	115.4 ± 201.1	458.2 ± 711.0	<0.0001	
TIMP1 (pg/ml)	183.5 ± 53.3	238.6 ± 106.1	<0.0001		189.7 ± 64.5	263.9 ± 113.8	<0.0001	
AOL/DSA	1.4 ± 1.2	10.9 ± 15.9	<0.0001	1.51 (1.07-2.15)	2.0 ± 2.6	18.3 ± 19.3	<0.0001	
MAL/DSA	10.6 ± 1.7	7.5 ± 3.4	<0.0001		10.2 ± 2.0	5.6 ± 3.4	<0.0001	0.52 (0.37-0.76)

95% CI: 0.77-0.99), HA (OR: 1.01, 95% CI: 1.01-1.02), and AOL/DSA (OR: 1.51, 95% CI: 1.07-2.15) were independently associated with the presence of significant fibrosis.

Comparison of Variables Associated with the Presence of Severe Fibrosis by Univariate and Multivariate Analysis. Variables associated with the presence of severe fibrosis were assessed by univariate and multivariate analysis (Table 2). The variables of age ($P = 0.001$), AST ($P < 0.0001$), ALT ($P < 0.0001$), GGT ($P < 0.0001$), bilirubin ($P = 0.005$), α2-MG ($P < 0.0001$), HA ($P < 0.0001$), TIMP1 ($P < 0.0001$), and AOL/DSA ($P < 0.0001$) were significantly higher in the severe fibrosis group than in the no severe fibrosis group. The variables albumin ($P < 0.0001$), cholinesterase ($P < 0.0001$), cholesterol ($P = 0.016$), platelets ($P < 0.0001$), prothrombin time ($P < 0.001$), and MAL/DSA ($P < 0.0001$) were significantly lower in the severe fibrosis group than in the no severe fibrosis group. Multivariate analysis showed that age (OR: 1.15, 95% CI: 1.02-1.31), platelets (OR: 0.74, 95% CI: 0.58-0.94), and MAL/DSA (OR: 0.52, 95% CI:

Table 3. Variables Associated with the Presence of Cirrhosis (F4) by Univariate and Multivariate Analysis

Features	No Cirrhosis (n=157)	Cirrhosis (n = 26)	*P* Value	Odds Ratio (95% CI) (Multivariate)
Age (years)	56.6 ± 11.7	63.8 ± 7.3	0.0016	
Male sex (%)	60 (38.2)	15 (57.7)	0.061	
AST (IU/L)	54.6 ± 41.7	74.9 ± 53.7	0.016	
ALT (IU/L)	62.1 ± 58.1	67.2 ± 48.2	0.446	
GGT (IU/L)	48.5 ± 63.9	64.9 ± 53.8	0.0031	
Bilirubin (mg/dL)	0.6 ± 0.3	1.0 ± 0.5	<0.0001	
Albumin (g/L)	4.2 ± 0.4	3.6 ± 0.5	<0.0001	
Cholinesterase (IU/L)	305.3 ± 83.9	181.7 ± 90.1	<0.0001	
Cholesterol (mg/dL)	178.4 ± 33.3	146.9 ± 29.8	<0.0001	
Platelets (10^9/L)	172 ± 54	106 ± 36	<0.0001	0.76 (0.58-0.99)
Prothrombin time (%)	88.7 ± 35.5	79.2 ± 16.1	0.0004	
α2-MG (g/L)	346.2 ± 131.6	416.9 ± 127.8	0.019	
HA (μg/L)	137.1 ± 215.7	617.4 ± 915.1	<0.0001	
TIMP1 (pg/ml)	196.4 ± 70.4	287.3 ± 126.6	<0.0001	
AOL/DSA	3.4 ± 7.1	24.0 ± 20.4	<0.0001	
MAL/DSA	9.8 ± 2.4	4.2 ± 2.8	<0.0001	0.67 (0.49-0.90)

0.37-0.76) were independently associated with the presence of severe fibrosis.

Comparison of Variables Associated with the Presence of Cirrhosis by Univariate and Multivariate Analysis. Variables associated with the presence of cirrhosis were assessed by univariate and multivariate analysis (Table 3). Age ($P = 0.0016$), AST ($P = 0.016$), GGT ($P = 0.0031$), bilirubin ($P < 0.0001$), α2-MG ($P = 0.019$), HA ($P < 0.0001$), TIMP1 ($P < 0.0001$), and AOL/DSA ($P < 0.0001$) were significantly higher in the cirrhosis group than in the no cirrhosis group. Albumin ($P < 0.0001$), cholinesterase ($P < 0.0001$), cholesterol ($P < 0.0001$), platelets ($P < 0.0001$), prothrombin time ($P = 0.0004$), and MAL/DSA ($P < 0.0001$) were significantly lower in the cirrhosis group than in the no cirrhosis group. Multivariate analysis showed that platelets (OR: 0.76, 95% CI: 0.58-0.99) and MAL/DSA (OR: 0.67, 95% CI: 0.49-0.90) were independently associated with the presence of cirrhosis.

Evaluation of the Two Glyco-Parameters AOL/DSA and MAL/DSA for Estimating the Progression of Liver Fibrosis. To assess the correlation of the two obtained glyco-parameters with the progression of fibrosis, we analyzed the data of triple lectins from HISCL measurements on the 183 CHC patients. The boxplots of AOL/DSA and MAL/DSA in relation to the fibrosis staging are shown in Fig. 1A,B, respectively. The AOL/DSA values gradually increased with the progression of fibrosis and Pearson's correlation efficient was R = 0.61. On the other hand, the MAL/DSA values gradually decreased with the progression of fibrosis and Pearson's correlation efficient was R = −0.69. Both parameters fitted the quantification of the progression of fibrosis from F2 to F4.

LecT-Hepa, Combined with Two Glyco-Parameters, Was Evaluated in the Diagnosis of Significant Fibrosis, Severe Fibrosis, and Cirrhosis. LecT-Hepa was calculated using two glyco-parameters (AOL/DSA and MAL/DSA). The boxplots of LecT-Hepa in relation to the fibrosis staging are shown in Fig. 2. The LecT-Hepa values gradually increased with the progression of fibrosis. Pearson's correlation coefficient between LecT-Hepa and liver fibrosis was very high (R = 0.72), and was superior to those for AOL/DSA (R = 0.61) and MAL/DSA (R = −0.69). We next examined AUC to characterize the diagnostic accuracy of LecT-Hepa at each stage of fibrosis, i.e., significant fibrosis (F2/F3/F4), severe fibrosis (F3/F4), and cirrhosis (F4). For the prediction of significant fibrosis, AUC (95% CI), sensitivity, specificity, PPV, NPV, LR (+), and LR (−) of the test were 0.802 (0.738-0.865), 59.6%, 89.9%, 85.7%, 66.7%, 5.89, and 0.45,

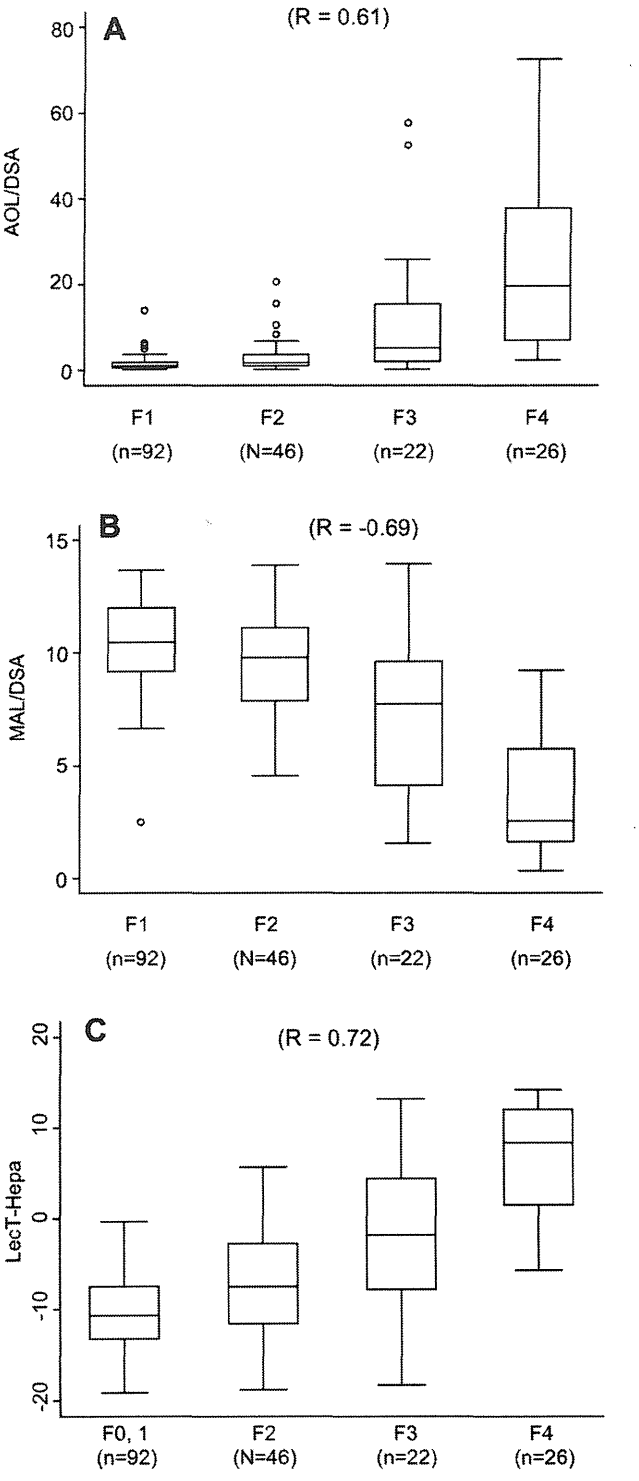

Fig. 1. Boxplot of (A) AOL/DSA, (B) MAL/DSA, and (C) LecT-Hepa in relation to the fibrosis score. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the dots represent outliers. The line across the box indicates the median value. Correlation of AOL/DSA, MAL/DSA, and LecT-Hepa was measured by HISCL with the progression of liver fibrosis. R: Pearson's correlation coefficient.