before LDLT. Additionally, the levels of three essential amino acids (valine, leucine and isoleucine), and one non-essential amino acid (glutamine) in patients with sarcopenia after LDLT were significantly decreased compared with before LDLT. This suggested a central role for skeletal muscle in the metabolism of amino acids or activation of the immune system in stressed conditions such as sepsis, and the importance of immunity induced by amino acids (e.g. glutamine) against sepsis.

Sarcopenia is one of the most common complications of a number of chronic diseases including chronic liver disease, chronic renal failure, chronic heart failure and a variety of malignancies.[6–8] Multiple mechanisms, such as alterations in sex hormones, imbalance between protein synthesis and breakdown, changes in physical activity and inadequate nutrition, are involved in the development of sarcopenia. As a nutritional intervention for sarcopenia, in this study we suggested the possibility of the effect of perioperative nutritional therapy on sarcopenia in patients with cirrhosis.

In this study, preoperative lower plasma glutamine levels were an independent risk factor of postoperative sepsis after LDLT. Associations with plasma glutamine levels and a life-threatening infection such as sepsis have been reported for patients after surgery or liver transplantation.[22,23] Glutamine has many important metabolic roles that protect or promote muscle mass and enhance the immune system.[17,24–27] Glutamine depletion decreases the proliferation of lymphocytes, which weakens the immune system.[27] It influences the cellular water content and has multiple effects on the immune system, intestinal functions and protein metabolism. Glutamine is also a precursor for the synthesis of glutathione, a powerful natural antioxidant enzyme that stimulates the formation of heat-shock proteins.[24–26,22,23,27] A deficiency of glutamine may promote fatal sepsis after LDLT, especially when patients, such as those with sarcopenia who have poor glutamine metabolism in skeletal muscle, are routinely treated with immunosuppressants.

We previously reported the effects of preoperative oral supplementation with BCAA of essential amino acids on postoperative bacteremia after LDLT. In conclusion, non-BCAA supplementation was an independent risk factor for postoperative sepsis. Racol, used for postoperative routine enteral nutrition administrated by feeding tube, included 17.52 g of proteins, 8.92 g of fat emulsion and 62.48 g of carbohydrate per pack (400 mL) and did not contain a nitrogen source as amino acids. Both glutamine and three essential amino acids combined as BCAA must be catabolized and produced from this abundant nutrition; however, there have been no reports comparing the impact of glutamine and BCAA for immunity against postoperative sepsis. The present study using multivariate analysis may provide useful information, as glutamine metabolism in skeletal muscle was superior for immunity against sepsis after LDLT when compared with BCAA. Nevertheless, this is a preliminary report, and further studies, such as randomized prospective studies, are necessary to clarify the beneficial effects of glutamine and BCAA supplementation on postoperative sepsis after LDLT. In terms of the required glutamine, catabolic wasting patients after LDLT should consider supplementary feeding with 2000 mg of glutamine per day, available as powder form p.o. or feeding tube. To date, we have routinely used an enteral nutrition formula that does not include glutamine, and thus patients with sarcopenia experience glutamine depletion.[10,15] Therefore, postoperative sepsis due to bacterial translocation may occur easily. This prospective study, now promoted in our institute, used early enteral nutrition with or without glutamine to demonstrate the impact of glutamine for the prevention of postoperative sepsis, referred to as sarcopenia.

In conclusion, the profile of various amino acids levels before and after LDLT indicated that low glutamine levels are an independent risk factor for postoperative sepsis after LDLT. There was a strong association between sarcopenia and glutamine metabolism; therefore, further research of postoperative early nutrition including glutamine should be performed to clarify the positive impact of glutamine protection against sepsis induced by sarcopenia.

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REFERENCES

1 Selberg O, Böttcher J, Tusch G, Pichlmayr R, Henkel E, Müller MJ. Identification of high- and low-risk patients before liver transplantation: a prospective cohort study of nutritional and metabolic parameters in 150 patients. *Hepatology* 1997; 25: 652–7.
2 Pikul J, Sharpe MD, Lowndes R, Ghent CN. Degree of preoperative malnutrition is predictive of postoperative morbidity and mortality in liver transplant recipients. *Transplantation* 1994; 57: 469–72.
3 Sanchez AJ, Aranda-Michel J. Nutrition for the liver transplant patient. *Liver Transpl* 2006; 12: 1310–6.
4 van Vledder MG, Levolger S, Ayez N, Verhoef C, Tran TC, Ijzermans JN. Body composition and outcome in patients

undergoing resection of colorectal liver metastases. *Br J Surg* 2012; 99: 550–7.
5 Sabel MS, Lee J, Cai S, Englesbe MJ, Holcombe S, Wang S. Sarcopenia as a prognostic factor among patients with stage III melanoma. *Ann Surg Oncol* 2011; 18: 3579–85.
6 Tan BH, Birdsell LA, Martin L, Baracos VE, Fearon KC. Sarcopenia in an overweight or obese patient is an adverse prognostic factor in pancreatic cancer. *Clin Cancer Res* 2009; 15: 6973–9.
7 Englesbe MJ, Patel SP, He K, *et al.* Sarcopenia and mortality after liver transplantation. *J Am Coll Surg* 2010; 211: 271–8.
8 Meza-Junco J, Montano-Loza AJ, Baracos VE, *et al.* Sarcopenia as a prognostic index of nutritional status in concurrent cirrhosis and hepatocellular carcinoma. *J Clin Gastroenterol* 2013; 47: 861–70.
9 Kaido T, Ogawa K, Fujimoto Y, *et al.* Impact of sarcopenia on survival in patients undergoing living donor liver transplantation. *Am J Transplant* 2013; 13: 1549–56.
10 Ikegami T, Shirabe K, Yoshiya S, *et al.* Bacterial sepsis after living donor liver transplantation: the impact of early enteral nutrition. *J Am Coll Surg* 2012; 214: 288–95.
11 Montano-Loza AJ, Meza-Junco J, Prado CM, *et al.* Muscle wasting is associated with mortality in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2012; 10: 166–73.
12 Masuda T, Shirabe K, Ikegami T, *et al.* Sarcopenia is a prognostic factor in living donor liver transplantation. *Liver Transpl* 2014; 20: 401–7.
13 Toshima T, Shirabe K, Fukuhara T, *et al.* Suppression of autophagy during liver regeneration impairs energy charge and hepatocyte senescence in mice. *Hepatology* 2014; 60: 290–300.
14 Yoshizumi T, Shirabe K, Nakagawara H, *et al.* Skeletal muscle area correlates with body surface area in healthy adults. *Hepatol Res* 2014; 44: 313–8.
15 Shirabe K, Yoshimatsu M, Motomura T, *et al.* Beneficial effects of supplementation with branched-chain amino acids on postoperative bacteremia in living donor liver transplant recipients. *Liver Transpl* 2011; 17: 1073–80.
16 Toshima T, Shirabe K, Ikegami T, *et al.* A novel serum marker, glycosylated Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA'-M2BP), for assessing liver fibrosis. *J Gastroenterol* 2014; 50: 76–84.
17 Holecek M, Sispera L, Skalska H. Enhanced glutamine availability exerts different effects on protein and amino acid metabolism in skeletal muscle from healthy and septic rats. *JPEN J Parenter Enteral Nutr* 2014; 46: 1377–84.
18 Wagenmakers AJ. Protein and amino acid metabolism in human muscle. *Adv Exp Med Biol* 1998; 441: 307–19.
19 Dickinson JM, Rasmussen BB. Amino acid transporters in the regulation of human skeletal muscle protein metabolism. *Curr Opin Clin Nutr Metab Care* 2013; 16: 638–44.
20 Taylor PM. Role of amino acid transporters in amino acid sensing. *Am J Clin Nutr* 2014; 99: 223S–30S.
21 Holecek M, Sispera L. Glutamine deficiency in extracellular fluid exerts adverse effects on protein and amino acid metabolism in skeletal muscle of healthy, laparotomized, and septic rats. *Amino Acids* 2014; 46: 1377–84.
22 Viggiano E, Passavanti MB, Pace MC, *et al.* Plasma glutamine decreases immediately after surgery and is related to incisiveness. *J Cell Physiol* 2012; 227: 1988–91.
23 Cohen J, Chin WD. Nutrition and sepsis. *World Rev Nutr Diet* 2013; 105: 116–25.
24 Doherty TJ. Invited review: aging and sarcopenia. *J Appl Physiol* 2003; 95: 1717–27.
25 Karinch AM, Pan M, Lin CM, Strange R, Souba WW. Glutamine metabolism in sepsis and infection. *J Nutr* 2001; 131: 2535S–8S.
26 Kraft G, Coate KC, Dardevet D, *et al.* Portal glucose delivery stimulates muscle but not liver protein metabolism. *Am J Physiol Endocrinol Metab* 2012; 303: E1202–11.
27 Walrand S. Ornithine alpha-ketoglutarate: could it be a new therapeutic option for sarcopenia? *J Nutr Health Aging* 2010; 14: 570–7.

SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found in the online version of this article at the publisher's web site.

Supplementary Table 1 Plasma amino acids values for assessment of postoperative sepsis.
Supplementary Figure 1

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GASTROINTESTINAL

Hepatocellular carcinoma: clinical significance of signal heterogeneity in the hepatobiliary phase of gadoxetic acid-enhanced MR imaging

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Abstract
Objectives To clarify the relationship between the biological behaviour of hepatocellular carcinomas (HCCs) and their signal intensity in the hepatobiliary phase of gadoxetic acid-enhanced MR imaging with a special focus on the signal heterogeneity.
Methods A total of 68 patients with 70 pathologically proven HCCs were enrolled. On the basis of the signal intensity in the hepatobiliary phase, the lesions were classified into three groups: group 1, homogeneous hypointensity (n=44); group 2, heterogeneous hyperintensity (n=20); and group 3, homogeneous hyperintensity (n=6). The clinicopathological findings were compared among the three groups.
Results The tumour size and the serum level of protein induced by vitamin K absence or antagonist-II (PIVKA-II) were significantly higher in group 2 compared to group 1 (p=0.0155, p=0.0215, respectively) and compared to group 3 (p=0.0330, p=0.0220, respectively). The organic anion transporting polypeptide 8 (OATP8) expression in group 2 and group 3 was significantly higher than in group 1 (p<0.0001, p<0.0001, respectively). Group 2 showed a significantly lower disease-free survival rate compared to group 1 (p=0.0125), and group 2 was an independent prognostic factor for disease-free survival (p=0.0308).
Conclusions HCCs in the hepatobiliary phase that are heterogeneously hyperintense on gadoxetic acid-enhanced MR imaging have more malignant potential than other types of HCCs.
Key Points
- *Heterogeneous uptake of gadoxetic acid suggests more malignant potential in HCC*
- *Uptake of gadoxetic acid does not suggest less malignancy in HCC*
- *Evaluation of signal heterogeneity on gadoxetic acid-enhanced MR imaging is useful*

Keywords Hepatocellular carcinoma · Hepatocarcinogenesis · Magnetic resonance imaging · Gadoxetic acid · Organic anion transporting polypeptide 8

Abbreviations

AFP	alpha-fetoprotein
HBP	hepatobiliary phase
HCC	hepatocellular carcinoma
MR	magnetic resonance
OATP	organic anion transporting polypeptide
PIVKA-II	protein induced by vitamin K absence or antagonist-II
ROI	region of interest

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common neoplasm in the world, and is especially common in East Asia and sub-Saharan Africa [1–3]. The accurate detection of HCC is one of the most important issues in the management of patients with chronic liver disease. Gadoxetic acid is a hepatobiliary-specific contrast medium for magnetic resonance (MR) imaging [4, 5]. It is taken up to varying degrees by functioning hepatocytes and is excreted in the bile. Malignant tumours such as HCC without functioning hepatocytes usually show hypointensity compared to background liver in the hepatobiliary phase (HBP) [4]. Gadoxetic acid-enhanced MR imaging has become an important imaging modality to diagnose HCC with high accuracy owing to its high lesion-to-liver contrast [6–8].

Some HCCs uptake gadoxetic acid and are recognized as iso- or hyperintense lesions in the HBP [9–11]. It has been reported that the expression of organic anion transporting polypeptide 8 (OATP8, synonymous with OATP1B3) in HCC, the uptake transporter of gadoxetic acid, determines the hyperintensity in the HBP [10, 11]. In addition, recent studies revealed that the difference of signal intensity in the HBP of HCC correlates with biological behaviours and patient outcomes [12–15]. Briefly, hyperintense HCCs in the HBP show lesser aggressive biological behaviour or more favourable outcome than hypointense HCCs. In those studies, HCCs were classified as either hypointense or hyperintense only.

However, in daily practice, we sometimes encounter HCCs which show heterogeneous intensity in the HBP. It was reported that some well- or moderately differentiated HCCs show increased expression of OATP8 and hyperintensity in the HBP [10, 11, 16], which suggests that HCCs which contain different tumour differentiation or different expressions of character (e.g. OATP8) may be heterogeneously hyperintense in the HBP. Other morphological characteristics of HCC such as tumour necrosis may modify the imaging findings in the HBP. These characteristics of HCCs may affect the biological behaviours and patient outcomes, but little is known about the clinical significance of HCCs that show heterogeneous intensity in the HBP. The purpose of the present study was to evaluate the signal intensity in the HBP of HCCs with a focus on their signal heterogeneity and to compare it with clinicopathological findings including OATP8 expression and prognoses.

Materials and methods

Patients

Our institutional review board approved this study, and the requirements for informed consent were waived for this retrospective study. Between June 2008 and December 2010, 99 patients underwent surgical resection of HCC at our institution. Patients were excluded if they had previous treatment (n=18) or if they did not have preoperative gadoxetic acid-enhanced MR imaging (n=5). Patients who were diagnosed as having recurrent HCC were also excluded (n=8). Finally, 68 patients with 70 new-onset HCC lesions were retrospectively enrolled in this study. The patients' details and tumour profiles are summarized in Table 1.

MR techniques

Gadoxetic acid-enhanced MR imaging was performed prior to surgical resection (mean time before surgery, 21.4±16.1 days [range, 2–102 days]). MR images were examined on a clinical 1.5- or 3.0-T MR system (Intera Achieva Nova Dual, 1.5 T, or Achieva TX, 3.0 T, Philips Healthcare, Best, the Netherlands) with a sensitivity encoding technique (SENSE) using a 16- or 32-channel phased-array coil. For the dynamic study, fat-suppressed gradient-echo T1-weighted images with a three-dimensional (3D) acquisition sequence (three-dimensional T1 high-resolution isotropic volume excitation [THRIVE] or enhanced THRIVE [eTHRIVE]) were obtained.

For the gadoxetic acid-enhanced MRI, a multiphase dynamic study including arterial dominant, portal, late and hepatobiliary phases was performed. The detailed imaging

Table 1 Patients' details and tumour profiles

Gender (male/female)	48/20
Age (years)*	68.7±11.5 (36–87)
Etiology of liver disease	
Hepatitis B virus	10
Hepatitis C virus	40
Alcoholism	8
Unknown	19
Background liver	
Normal	12
Chronic hepatitis	39
Cirrhosis	17
Child–Pugh class	
A	66
B	2
C	0
AFP (ng/ml)*	20,873.6±86,266.6 (1.0–577,660)
PIVKA-II (mAU/ml)*	4,524.9±14,500.8 (2.0–75,000)
No. of nodules	
Single	66
Two	2
Tumour size (cm)*	4.0±3.2 (0.5–12.6)

*Data are mean±standard deviation

Ranges are in parentheses

parameters of THRIVE in the 1.5-T MR system were as follows: repetition time/echo time=3.0 ms/1.0 ms, flip angle 20°, matrix 224×116, field of view 36 cm, slice thickness/gap=4 mm/−2 mm, SPAIR, scan time 18 s, and breath-holding. The detailed imaging parameters of cTHRIVE in the 3.0-T MR system were as follows: repetition time/echo time=3.0 ms/1.4 ms, flip angle 10°, matrix 252×200, field of view 36 cm, slice thickness/gap=3 mm/−1.5 mm, SPAIR, imaging time 17.9 s, and breath-holding.

The total amount of gadoxetic acid (Primovist: Bayer, Osaka, Japan) based on the patient's body weight (0.025 mmol/kg) was intravenously injected for 5 s [17, 18], followed by a 20-ml physiological saline flush using an automatic injector (Nemoto Kyourindo, Tokyo). We used the test injection method to determine the optimal scan timing of the arterial dominant phase referring to a previous report [19]. A test dose of 0.5 ml of gadoxetic acid was injected and flushed with 20 ml of physiological saline at the same injection rate. The scanning of the portal, late and hepatobiliary phases began at the arterial dominant phase +30 s, 180 s and 20 min after the injection of the contrast agent, respectively.

Image analysis

Images of all axial sections of the lesions were evaluated by two radiologists independently (N.F. and K.M. with 11 and 8 years of experience in abdominal imaging, respectively) without information regarding the clinical and pathological results. Hyperintensity in the HBP was qualitatively defined as higher signal intensity than that in the precontrast image [9]. If tumour necrosis was suspected, we simply compared the signal intensity in the HBP to that of the precontrast image. On the basis of these criteria, each lesion was classified into one of the three groups: group 1, homogeneous hypointensity in the HBP; group 2, heterogeneous hyperintensity in the HBP; and group 3, homogeneous hyperintensity in the HBP. Interobserver agreement between the two radiologists was evaluated using weighted κ statistics, with a κ value of less than 0.20 indicating poor agreement, 0.20–0.39 fair agreement, 0.40–0.59 moderate agreement, 0.60–0.79 substantial agreement, and more than 0.80 excellent agreement. After evaluating the results in a consensus fashion, data analysis was performed. Arterial enhancement was defined as higher intensity relative to the surrounding liver parenchyma in the arterial phase of the dynamic MR examination. The presence of arterial enhancement of each lesion was also evaluated by one radiologist (A.N. with 20 years of experience in abdominal imaging).

Pathological diagnosis

The resected specimens were fixed in 10 % formalin, cut into 3-μm sections and stained with haematoxylin–eosin. The histological diagnosis of HCC was evaluated on the basis of the classification proposed by the World Health Organization [20] and the International Consensus Group for Hepatocellular Neoplasia [21].

We evaluated histological features such as tumour size, the dominant differentiation grade (well, moderately and poorly differentiated), capsule formation, infiltration to the capsule, septal formation, portal venous invasion, hepatic venous invasion and intrahepatic metastasis. Tumour size was defined as the largest diameter of the tumour in the resected specimen. We also evaluated the presence of different differentiated components or tumour necrosis in each lesion because these may affect the imaging findings of HCCs.

Immunohistochemical staining of OATP 8

Paraffin-embedded tissue sections (4 μm) containing sufficient tumour tissue were used for the study. Immunohistochemical staining was performed using primary antibody to OATP8 (mouse monoclonal antibody; NB100-74482; Novus Biologicals, Littelton, CO; 1:100 dilution) by the streptavidin–biotin–peroxidase method (Histofine SAB-PO Kit; Nichirei, Tokyo).

We evaluated the expression of OATP8 as described elsewhere [10]. The expression of OATP8 on the tumour cellular membrane in comparison with that of the surrounding non-neoplastic hepatocytes was semiquantitatively evaluated as follows: grade 0, no expression; grade 1, decreased expression; grade 2, equivalent expression; and grade 3, increased expression.

All of the pathological and immunohistochemical evaluations were performed by two pathologists (Y.K. and S.A. with 4 and 14 years of experience in liver pathology, respectively) in a consensus fashion without any knowledge of the clinicopathological findings.

Disease-free survival rate of the HCC patients

We were able to obtain prognostic data of 57 of the 68 patients who were followed up at our institution. Two patients were excluded because the surgical margin was affected by the carcinoma cells. We thus analysed the disease-free survival rate of 55 patients. After the initial operation, ultrasound and dynamic computed tomography were performed every 3 months in addition to a monthly measurement of alpha-fetoprotein (AFP). The median follow-up period was 643 days (range 59–1,518 days). We compared the disease-free survival rates of the three lesion groups.

Statistical analysis

We used Fisher's exact test to analyse the correlation between the signal intensity pattern in the HBP and clinicopathological

factors (gender, origin of liver disease, background liver, Child–Pugh class, tumour differentiation, capsule formation, infiltration to the capsule, septal formation, portal venous invasion, hepatic venous invasion, intrahepatic metastasis, mixed differentiation and tumour necrosis) or the Kruskal–Wallis test and Mann–Whitney *U* test (age, serum level of alpha-fetoprotein (AFP), serum level of protein induced by vitamin K absence or antagonist-II (PIVKA-II), tumour size, OATP8 expression). First, we compared these factors among the three lesion groups. If a significant difference was obtained, we compared the factor between each pair of groups. We evaluated the patient disease-free survival using the Kaplan–Meier method with the log-rank test. We divided the continuous values of clinicopathological factors by their median values, and all variables found to be significant in a univariate analysis were entered into the Cox proportional hazard model for a multivariate analysis. JMP 9.0.2 software (SAS Institute, Cary, NC) was used for the analysis. *P* values less than 0.05 were considered significant.

Results

Imaging pattern in HBP and clinicopathological findings

Of the 70 HCCs in the HBP evaluated by their intensity on gadoxetic acid-enhanced MR imaging, 44 HCCs (62.9 %) showed homogeneous hypointensity (group 1), 20 HCCs (28.6 %) showed heterogeneous hyperintensity (group 2) and six HCCs (8.6 %) showed homogeneous hyperintensity (group 3). The weighted κ value representing interobserver agreement was 0.86, and excellent agreement was obtained. Arterial enhancement was observed in 65 (92.6 %) of 70 HCCs. The correlations between the signal intensity pattern in the HBP and clinicopathological findings are summarized in Tables 2 and 3.

The serum level of the PIVKA-II and the tumour size were significantly different among the three lesion groups (p=0.0322 and p=0.0237, respectively). The two-group comparisons revealed that the serum PIVKA-II levels of group 2 were significantly higher than those of both group 1 and group 3 (p=0.0215 and p=0.0220, respectively). In addition, the tumour sizes of group 2 were significantly larger than those of group 1 and group 3 (p=0.0155 and p=0.0330, respectively). The serum PIVKA-II level and tumour size were not significantly different between group 1 and group 3 (Fig. 1a, b).

Of the 44 HCCs of group 1, the dominant differentiation was well-differentiated HCC in four cases (9.1 %), moderately differentiated HCC in 29 cases (65.9 %) and poorly differentiated HCC in 11 cases (25 %). Of the 20 HCCs of group 2, the dominant differentiation was well-differentiated HCC in two cases (10.0 %), moderately differentiated HCC in 15 cases (75.0 %) and poorly differentiated HCC in three cases (15.0 %). Of the six HCCs in group 3, the dominant differentiation was well-differentiated HCC in one case (16.7 %) and moderately differentiated HCC in five cases (83.3 %). The dominant differentiation was not different among the three lesion groups.

The frequency of the presence of different differentiated components (mixed differentiation) and that of tumour necrosis were not significantly different among the three lesion groups.

Table 2 Correlation between clinical factors and the signal intensity pattern in the HBP of 68 patients of HCC

	Group 1 (n=43)	Group 2 (n=19)	Group 3 (n=6)	p Value
Sex (male/female)	30/13	14/5	4/2	0.9295
Age (years)*	67.5±12.4	62.5±8.1	73.2±8.7	0.0523
Etiology of liver disease				
Hepatitis B virus	8	1	1	0.4134
Hepatitis C virus	24	11	5	0.5175
Alcoholism	3	4	1	0.2177
Background liver				0.2858
Normal	7	5	0	
Chronic hepatitis	24	11	4	
Cirrhosis	12	3	2	
Child–Pugh class				0.5542
A	41	19	6	
B	2	0	0	
C	0	0	0	
α-fetoprotein (ng/ml)*	27,750.0±106,402.8	11,898.3±30,668.5	14.2±15.8	0.2117
PIVKA-II (mAU/ml)*	3,344.7±12,099.7	8,601.3±20,337.2	75.3±119.2	0.0322

*Data are mean±standard deviation

Table 3 Correlation between pathological factors and the signal intensity pattern in the HBP of 70 HCCs

	Group 1 (n=44)	Group 2 (n=20)	Group 3 (n=6)	p Value
Tumour size (cm)*	3.5±2.8	5.5±3.8	2.4±1.2	0.0237
Differentiation (dominant)				0.6248
Well	4	2	1	
Moderate	29	15	5	
Poor	11	3	0	
Mixed differentiation (%)	59.1	40.0	33.3	0.2797
Capsule formation (%)	59.1	75.0	33.3	0.1845
Infiltration to the capsule (%)	50.0	55.0	33.3	0.7107
Septal formation (%)	63.6	70.0	33.3	0.2715
Portal venous invasion (%)	29.6	55.0	16.7	0.1024
Hepatic venous invasion (%)	22.7	25.0	16.7	0.9126
Intrahepatic metastasis (%)	9.1	0	0	0.5151
Tumour necrosis (%)	30.2	31.6	0	0.4158

*Data are mean±standard deviation

Imaging pattern in the HBP and OATP8 expression

The immunohistochemical analysis revealed that the expression of OATP8 differed significantly among the three groups (p<0.0001). The OATP8 expression in both group 2 and group 3 was significantly higher than that in group 1 (p<0.0001 and p<0.0001, respectively). However, the OATP8 expression was not significantly different between group 2 and group 3 (p=0.1227) (Fig. 2).

Three cases that are typical of the lesion groups are shown in Figs. 3, 4 and 5.

Outcome after HCC surgery

Each of the 55 patients had only one HCC. Thirty-five patients had HCC in group 1, 14 patients had HCC in group 2 and six patients had HCC in group 3. The 1- and 3-year disease-free survival rates were 80.2 % and 68.0 % in the patients in group 1, 69.6 % and 10.2 % in group 2, and 100 % and 66.7 % in group 3, respectively (Fig. 6). The patients in group 2 showed significantly poorer disease-free survival than the patients in group 1 (p=0.0125). The patients in group 2 tended to show poorer disease-free survival than the patients in group 3 (p=0.0650), but no significant difference was obtained. The disease-free survival rate showed no significant difference between patients in group 1 and group 3 (p=0.6073).

Univariate and multivariate analysis of disease-free survival in HCC patients

The univariate analysis of the patients in group 1 and group 2 revealed the prognostic factors of group 2 lesions, namely high serum PIVKA-II level (≥84.0 mAU/ml), tumour size (≥2.9 cm), capsule formation, capsule infiltration, portal venous invasion, hepatic venous invasion and intrahepatic metastasis. The multivariate analysis including these factors revealed the following independent factors for disease-free survival: group 2 (p=0.0308), intrahepatic metastasis (p=0.0016) and the serum PIVKA-II level (p=0.0489) (Table 4).

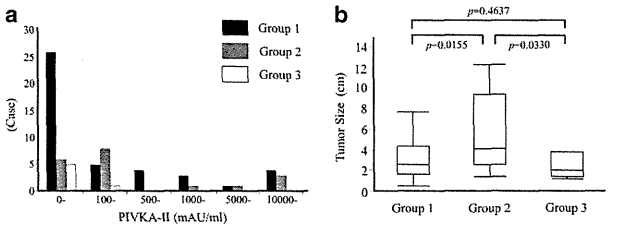

Fig. 1 Correlations between the signal intensity pattern in HBP and PIVKA-II or tumour size. **a** The serum PIVKA-II levels of group 2 were significantly higher than those of group 1 and group 3 (p=0.0215, p=0.0220, respectively). There was no significant difference between group 1 and group 3. **b** The tumour sizes of group 2 were significantly larger than those of group 1 and group 3 (p=0.0155, p=0.0330, respectively). There was no significant difference in tumour size between group 1 and group 3

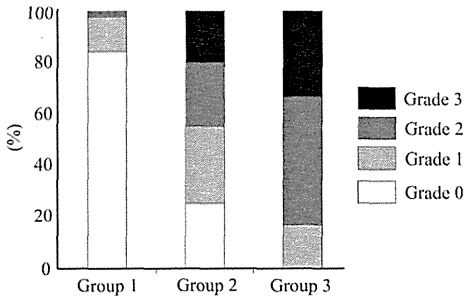

Fig. 2 The immunohistochemical expression of OATP8. The OATP8 expression in both group 2 and group 3 was significantly higher than that in group 1 ($p<0.0001$ and $p<0.0001$, respectively). There was no significant difference between group 2 and group 3 ($p=0.1227$)

Discussion

Gadoxetic acid-enhanced MR imaging is now an important imaging modality to diagnose HCC [6–8]. In addition to the high accuracy of the diagnosis of HCC, recent studies revealed that the imaging findings of HCC in the HBP may be a useful biomarker to indicate the malignant potential of HCC [12–15]. It was reported that hyperintense HCCs in the HBP show a higher grade of differentiation [12], smaller tumour sizes [13], a lower frequency of vascular invasion [12, 15], lower serum levels of AFP and PIVKA-II [12, 13], and lower recurrence rate [12, 13] compared to hypointense HCCs in the HBP. These studies suggest that hyperintense HCCs in the HBP show less aggressive biological behaviour and/or more favourable outcomes than hypointense HCCs in the HBP.

In our study, 20 HCCs (28.6 %) showed heterogeneous hyperintensity in the HBP (group 2). Such HCCs showed higher serum PIVKA-II levels and larger tumour sizes compared to the other HCCs. The serum PIVKA-II level and tumour size correlate with the histological degree of malignancy and the prognosis of HCC [22, 23]. In addition, the patients in group 2 showed poorer disease-free survival than patients in group 1, and lesion of group 2 was an independent prognostic factor for disease-free survival. These results seem to be different from those of the previous reports.

We suspect that the reason for this difference is the method of evaluation of heterogeneous lesions. Kitao et al. quantitatively divided HCCs into those showing hypointensity and hyperintensity compared to the signal intensity of the background liver in the HBP. The region of interest (ROI) was determined as the maximum area at the level of the largest diameter of the tumour for both nodules with homogeneous lesions and nodules with heterogeneous lesions [10]. Choi et al. divided HCCs into those showing hypointensity and iso- or hyperintensity quantitatively by an average of three ROI in the HCCs. For heterogeneous lesions, the ROIs in more homogeneous areas were chosen [14].

Kim et al. classified HCCs in the HBP as hyperintense or hypointense by visual assessment. For a heterogeneous lesion, they considered an HCC showing iso- or hyperintensity at

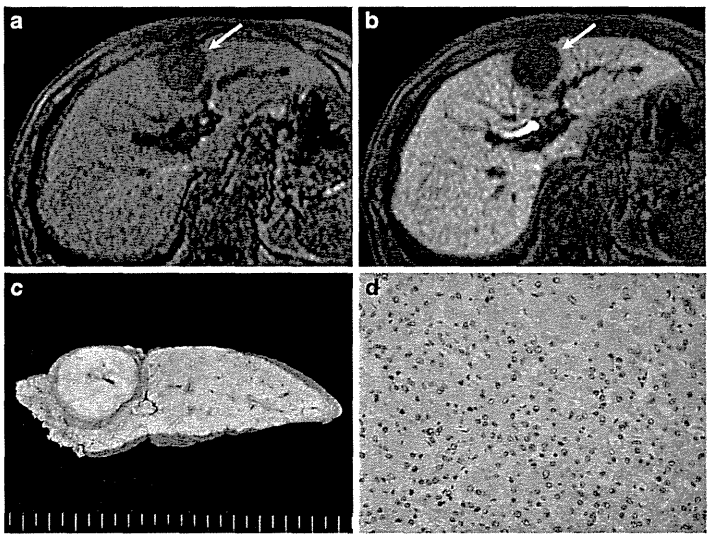

Fig. 3 A 66-year-old man with poorly differentiated HCC in the left lobe of the liver (*arrow*). Gadoxetic acid-enhanced imaging shows hypointensity in the precontrast scan (**a**) and homogeneous hypointensity in the hepatobiliary phase (i.e. group 1) (**b**). Surgically resected specimen shows a whitish nodule (**c**). Immunohistochemically, the tumour cells show no OATP8 expression (grade 0) (**d**). Original magnification×200

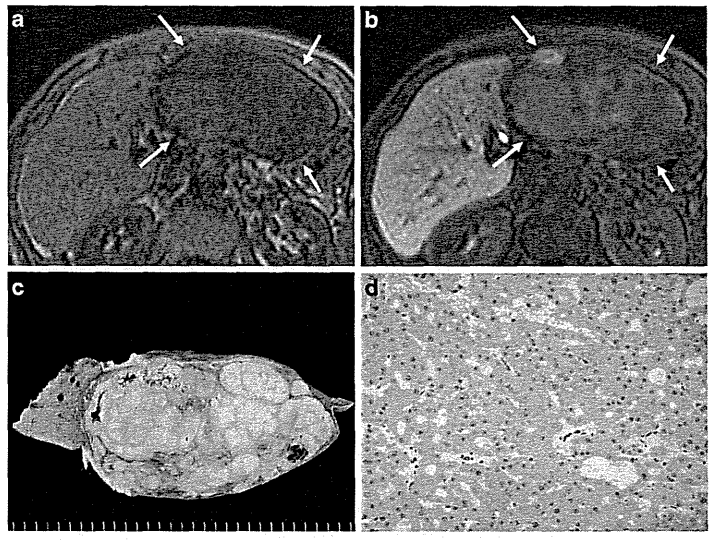

Fig. 4 A 76-year-old man with moderately differentiated HCC in the left lobe of the liver (*arrow*). Gadoxetic-enhanced MR imaging shows hypointensity in the precontrast scan (**a**) and heterogeneous hyperintensity in the hepatobiliary phase (i.e. group 2) (**b**). Surgically resected specimen shows a mixed greenish and whitish area (**c**). Immunohistochemically, the tumour cells show decreased expression of OATP8 (grade 1) (**d**). Original magnification×200

more than two-thirds of the tumour volume as a hyperintense HCC [15]. Indeed, they described the lack of an analysis of heterogeneous lesions in the HBP as a limitation [10, 13]. However, to the best of our knowledge, no studies have focused on the significance of heterogeneous lesions. Herein we present the clinical significance of signal heterogeneity; that is, heterogeneously hyperintense HCCs in the HBP have greater malignant potential than other HCCs. In other words, the uptake of gadoxetic acid does not always suggest less malignancy.

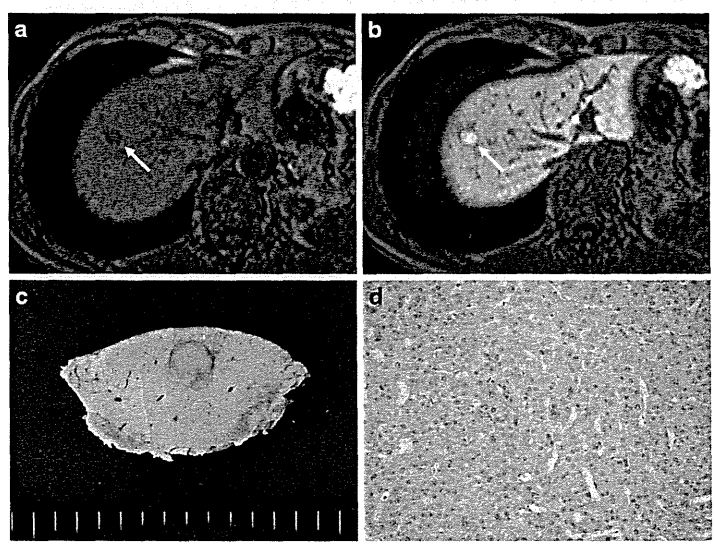

Fig. 5 A 65-year-old man with moderately differentiated HCC in the right lobe of the liver (*arrow*). Gadoxetic-enhanced MR imaging shows hypointensity in the precontrast scan (**a**) and homogeneous hyperintensity in the hepatobiliary phase (i.e. group 3) (**b**). Surgically resected specimen shows a greenish nodule (**c**). Immunohistochemically, the tumour cells show increased expression of OATP8 (grade 3) (**d**). Original magnification×200

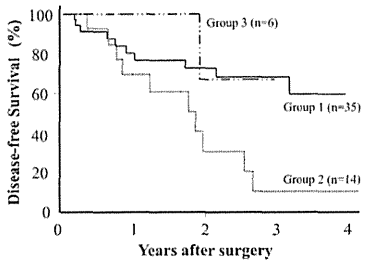

Fig. 6 The disease-free survival curves of the patients in the three lesion groups. The patients in group 2 showed significantly poorer disease-free survival than the patients in group 1 (p=0.0125). The patients in group 2 tended to show poorer disease-free survival than the patients in group 3 (p=0.0650), but no significant difference was obtained. The disease-free survival rate showed no significant difference between group 1 and group 3 (p=0.6073)

The mechanisms by which such a difference occurs within a single HCC are still controversial. Most HCCs without functioning hepatocytes usually show hypointensity in the HBP [4, 5], but some well- or moderately differentiated HCCs show an increased expression of OATP8 and hyperintensity in the HBP [9, 15, 16]. In the present study as well, all six of the HCCs (8.6 %) which showed homogeneous hyperintensity in the HBP (group 3) were well- or moderately differentiated HCCs, not poorly differentiated HCCs. Group 2 showed higher serum PIVKA-II levels and larger tumour sizes than group 1 and group 3. In addition, the OATP8 expression in both group 2 and group 3 was significantly higher than that of group 1. Several reports indicated that the OATP8 expression in HCCs is regulated by transcriptional factors such as hepatocellular nuclear factor (HNF) 1α [24], HNF 3β [25] and farnesoid X [26]. We hypothesize that HCCs showing homogeneous hypointensity (group 1) or homogeneous hyperintensity (group 3) in the HBP can become heterogeneous (group 2) in accordance with the tumour's development. However, further investigations to identify the mechanisms that control signal intensity in the HBP are needed.

Table 4 Univariate and multivariate analysis of disease-free survival in hepatocellular carcinoma of group 1 and 2

Variables	Hazard ratio	95 % CI	p value
Univariate analysis			
Group 2	2.9700	1.2005–7.3608	0.0192
PIVKA-II (≥84.0 mAU/ml)	15.5802	3.2339–279.8254	<0.0001
Tumour size (≥2.9 cm)	3.0610	1.1844–9.4196	0.0199
Capsule formation	3.8538	1.2909–16.5308	0.0134
Capsule infiltration	4.0173	1.5386–12.4682	0.0038
Portal venous invasion	4.4763	1.7868–12.6919	0.0012
Hepatic venous invasion	4.0326	1.6137–10.2294	0.0033
Intrahepatic metastasis	17.0095	5.0037–66.6595	<0.0001
Multivariate analysis			
Group 2	3.3686	1.1192–10.6465	0.0308
PIVKA-II (≥84.0 mAU/ml)	8.0887	1.0095–172.4858	0.0489
Intrahepatic metastasis	9.0781	2.3040–42.0477	0.0016

CI confidence interval

It has been reported that the tumour size was correlated with the prognosis of HCC [23]. Although tumour size was not an independent prognostic factor in our study population, the possibility that tumour size was correlated with disease-free survival cannot be denied. That is to say, the larger HCCs may tend to show a heterogeneous appearance and affect the prognosis. However, the present results at least provide evidence that HCCs which take up gadoxetic acid do not always have low malignant potential as previously reported [12–15]. It has been also reported that HCCs with an uptake of gadoxetic acid are significantly correlated with macroscopic green HCCs [9]. Although we did not evaluate the colour of the resected tumour, we consider that green HCCs may show varying degrees of colour and have components of another colour. It is not precise to classify HCC into only hyperintense or hypointense, as well as green HCC or not; a more detailed classification is needed. To obtain better evidence about the clinical impact of signal heterogeneity in the HBP, randomized studies of tumour size are needed.

In the present study, hyperintensity in the HBP was qualitatively defined as higher signal intensity than in the precontrast scan. The method used to define hyperintensity was different from those of the previous reports [10, 12–14]. To define hyperintensity in the HBP, those studies' authors compared the signal intensity of the lesions to that of the background liver. However, the signal intensity of the surrounding liver parenchyma was affected by degree of liver function [27–29] or by liver fibrosis [30, 31]. Thus, we consider that our method was more precise to evaluate the uptake of gadoxetic acid. Indeed, in some studies, the signal intensity of the lesion was also measured comparing the signal intensity in the precontrast scan and in the HBP [11, 15].

There were some limitations in this study. First, it was a qualitative evaluation; a quantitative evaluation was not performed. However, it is very difficult to quantify signal heterogeneity (e.g. the proportion of hyperintense area, and the uptake level of gadoxetic acid in each area) precisely. It may be practical to evaluate the signal intensity by visual assessment, which may be readily available to most clinicians. Second, the patient population was relatively small. A larger number of cases would make the findings of this study more reliable, especially regarding the HCCs of group 3. The small case number may be the reason why a significant difference was not obtained with regard to malignant potential between group 1 and group 3. Third, there was a variability of the imaging parameters because the MRIs were performed on two

different scanners (1.5- or 3.0-T MR). Finally, we cannot directly explain the reason for the correlation between signal heterogeneity and tumour malignancy. Further investigations, such as analyses of transcription factors of OATP8, are needed.

In conclusion, heterogeneously hyperintense HCCs in the HBP shown on gadoxetic acid-enhanced MR imaging have greater malignant potential than other HCCs. The evaluation of signal heterogeneity in the HBP on gadoxetic acid-enhanced MR imaging is useful for predicting aggressive behaviour in HCCs.

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References

1. Okuda K (1997) Hepatocellular carcinoma: clinicopathological aspects. J Gastroenterol Hepatol 12:314–318
2. Bosch FX, Ribes J, Díaz M, Cléries R (2004) Primary liver cancer: worldwide incidence and trends. Gastroenterology 127:5–16
3. Llovet JM, Burroughs A, Bruix J (2003) Hepatocellular carcinoma. Lancet 362:1907–1917
4. Vogl TJ, Kümmel S, Hammerstingl R et al (1996) Liver tumors: comparison of MR imaging with Gd-EOB-DTPA and Gd-DTPA. Radiology 200:59–67
5. Huppertz A, Balzer T, Blakeborough A, European EOB Study Group et al (2004) Improved detection of focal liver lesions at MR imaging: multicenter comparison of gadoxetic acid-enhanced MR images with intraoperative findings. Radiology 230:266–275
6. Ahn SS, Kim MJ, Lim JS, Hong HS, Chung YE, Choi JY (2010) Added value of gadoxetic acid-enhanced hepatobiliary phase MR imaging in the diagnosis of hepatocellular carcinoma. Radiology 255:459–466
7. Ichikawa T, Saito K, Yoshioka N et al (2010) Detection and characterization of focal liver lesions: a Japanese phase III, multicenter comparison between gadoxetic acid disodium-enhanced magnetic resonance imaging and contrast-enhanced computed tomography predominantly in patients with hepatocellular carcinoma and chronic liver disease. Invest Radiol 45:133–141
8. Golfieri R, Renzulli M, Lucidi V, Corcioni B, Trevisani F, Bolondi L (2011) Contribution of the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI to dynamic MRI in the detection of hypovascular small (≤2 cm) HCC in cirrhosis. Eur Radiol 21:1233–1242
9. Asayama Y, Tajima T, Nishie A et al (2011) Uptake of Gd-EOB-DTPA by hepatocellular carcinoma: radiologic-pathologic correlation with special reference to bile production. Eur J Radiol 80: e243–e248
10. Kitao A, Zen Y, Matsui O et al (2010) Hepatocellular carcinoma: signal intensity at gadoxetic acid-enhanced MR imaging–correlation with molecular transporters and histopathologic features. Radiology 256:817–826
11. Narita M, Hatano E, Arizono S et al (2009) Expression of OATP1B3 determines uptake of Gd-EOB-DTPA in hepatocellular carcinoma. J Gastroenterol 44:793–798
12. Kitao A, Matsui O, Yoneda N et al (2012) Hypervascular hepatocellular carcinoma: correlation between biologic features and signal intensity on gadoxetic acid-enhanced MR images. Radiology 265: 780–789
13. Choi JW, Lee JM, Kim SJ et al (2013) Hepatocellular carcinoma: imaging patterns on gadoxetic acid-enhanced MR images and their value as an imaging biomarker. Radiology 267:776–786
14. Choi JY, Kim MJ, Park YN et al (2011) Gadoxetate disodium-enhanced hepatobiliary phase MRI of hepatocellular carcinoma: correlation with histological characteristics. AJR Am J Roentgenol 197:399–405
15. Kim JY, Kim MJ, Kim KA, Jeong HT, Park YN (2012) Hyperintense HCC on hepatobiliary phase images of gadoxetic acid-enhanced MRI: correlation with clinical and pathological features. Eur J Radiol 81:3877–3882
16. Kitao A, Matsui O, Yoneda N et al (2011) The uptake transporter OATP8 expression decreases during multistep hepatocarcinogenesis: correlation with gadoxetic acid enhanced MR imaging. Eur Radiol 21:2056–2066
17. Nishie A, Asayama Y, Ishigami K et al (2014) Clinicopathological significance of the peritumoral decreased uptake area of gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid in hepatocellular carcinoma. J Gastroenterol Hepatol 29:561–567
18. Takayama Y, Nishie A, Nakayama T et al (2012) Hypovascular hepatic nodule showing hypointensity in the hepatobiliary phase of gadoxetic acid-enhanced MRI in patients with chronic liver disease: prediction of malignant transformation. Eur J Radiol 81:3072–3078
19. Shinozaki K, Yoshimitsu K, Irie H et al (2004) Comparison of test-injection method and fixed-time method for depiction of hepatocellular carcinoma using dynamic steady-state free precession magnetic resonance imaging. J Comput Assist Tomogr 28:628–634
20. Theise ND, Ishak KG, Kojiro M et al (2010) Hepatocelluilar carinoma. In: Bosman FT, Carneiro F, Hruban RH, Theise ND (eds) World Health Organization classification of the digestive system. IARC, Lyon, pp 205–216
21. International Consensus Group for Hepatocellular Neoplasia (2009) Pathologic diagnosis of early hepatocellular carcinoma: a report of the International Consensus Group for Hepatocellular Neoplasia. Hepatology 49:658–664
22. Miyaaki H, Nakashima O, Kurogi M, Eguchi K, Kojiro M (2007) Lens culinaris agglutinin-reactive alpha-fetoprotein and protein induced by vitamin K absence II are potential indicators of a poor prognosis: a histopathological study of surgically resected hepatocellular carcinoma. J Gastroenterol 42:962–968
23. Nanashima A, Abo T, Tobinaga S et al (2010) Relationship between period of survival and clinicopathological characteristics in patients with hepatocellular carcinoma who underwent hepatectomy. Hepatogastroenterology 57:540–546
24. Jung D, Kullak-Ublick GA (2003) Hepatocyte nuclear factor 1 alpha: a key mediator of the effect of bile acids on gene expression. Hepatology 37:622–631
25. Vavricka SR, Jung D, Fried M, Grützner U, Meier PJ, Kullak-Ublick GA (2004) The human organic anion transporting polypeptide 8 (SLCO1B3) gene is transcriptionally repressed by hepatocyte nuclear factor 3beta in hepatocellular carcinoma. J Hepatol 40:212–218

26. Jung D, Podvinec M, Meyer UA et al (2002) Human organic anion transporting polypeptide 8 promoter is transactivated by the farnesoid X receptor/bile acid receptor. Gastroenterology 122:1954–1966
27. Verloh N, Haimerl M, Zeman F et al (2014) Assessing liver function by liver enhancement during the hepatobiliary phase with Gd-EOB-DTPA-enhanced MRI at 3 Tesla. Eur Radiol 24:1013–1019
28. Yoneyama T, Fukukura Y, Kamimura K et al (2014) Efficacy of liver parenchymal enhancement and liver volume to standard liver volume ratio on Gd-EOB-DTPA-enhanced MRI for estimation of liver function. Eur Radiol 24:857–865
29. Nishie A, Ushijima Y, Tajima T et al (2012) Quantitative analysis of liver function using superparamagnetic iron oxide- and Gd-EOB-DTPA-enhanced MRI: comparison with technetium-99m galactosyl serum albumin scintigraphy. Eur J Radiol 81:1100–1104
30. Verloh N, Haimerl M, Rennert J et al (2013) Impact of liver cirrhosis on liver enhancement at Gd-EOB-DTPA enhanced MRI at 3 Tesla. Eur J Radiol 82:1710–1715
31. Nishie A, Asayama Y, Ishigami K et al (2012) MR prediction of liver fibrosis using a liver-specific contrast agent: superparamagnetic iron oxide versus Gd-EOB-DTPA. J Magn Reson Imaging 36:664–671

Sarcopenia is a prognostic factor for overall survival in patients with critical limb ischemia

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Background: **Sarcopenia has been proposed as a prognostic factor for various diseases. Patients with critical limb ischemia (CLI) have a very poor prognosis, but sarcopenia has not been reported as a prognostic factor for CLI patients. If sarcopenia is associated with the prognosis of CLI patients, it could help select the treatment plan. Therefore, we examined whether sarcopenia is a prognostic factor for CLI patients.**

Methods: **We performed a retrospective study of CLI patients diagnosed with Fontaine III or IV peripheral artery disease who underwent preoperative computed tomography imaging and revascularization between January 2002 and December 2009. The presence of sarcopenia was defined as skeletal muscle area of <114.0 cm^2 for men or <89.8 cm^2 for women using transverse computed tomography scans at the third lumbar vertebra. We compared the 5-year survival rate and clinical characteristics between patients with or without sarcopenia. We also screened possible prognostic factors for overall survival using hazard ratios (HRs) with 95% confidence intervals (CIs).**

Results: **Of 64 eligible patients, 28 patients had sarcopenia and 36 did not. There were significant differences in age, skeletal muscle area, body mass index, and the presence of smoking, cerebrovascular disease, and hemodialysis between patients with and without sarcopenia (all $P < .05$). The 5-year survival rate was significantly lower in patients with sarcopenia (23.5% vs 77.5%, $P = .001$). Prognostic factors for overall survival were the presence of sarcopenia (HR, 3.22; 95% CI, 1.24-9.11; $P = .02$), requirement for hemodialysis (HR, 4.30; 95% CI, 1.60-12.2; $P = .004$), and postoperative complications (HR, 5.02; 95% CI, 1.90-13.7; $P = .001$).**

Conclusions: **Our results suggest that sarcopenia is a prognostic factor for CLI patients. Exercise and nutritional interventions focusing on improving sarcopenia might be useful treatment options for CLI patients. (J Vasc Surg 2015;61:945-50.)**

The Bypass vs Angioplasty in Severe Ischaemia of the Leg (BASIL) study showed that bypass is an appropriate treatment for critical limb ischemia (CLI) patients with an expected survival of >2 years.[1] Some prognostic factors for CLI have been suggested during the past 15 years.[2-13] Meanwhile, several recent studies have suggested that sarcopenia, a reduction in skeletal muscle, is a prognostic factor for several diseases.[14-21] However, no studies have assessed whether sarcopenia is a prognostic factor for CLI patients.

The prognosis of CLI patients is poor, and the risk of cardiovascular and cerebrovascular events is high.[8,22] However, the relationship between sarcopenia and the prognosis of vascular disease is unclear. Sarcopenia is relatively easy to diagnose by preoperative computed tomography (CT) imaging. Therefore, if sarcopenia could predict the prognosis of CLI patients, it might help clinicians select the most appropriate treatment plan. In addition, if the mechanism underlying the association between sarcopenia and prognosis could be determined, treatments for sarcopenia, such as exercise, might improve the prognosis of CLI patients. From this context, the aim of this study was to determine the clinical significance of sarcopenia as a prognostic factor for CLI patients.

METHODS

The Kyushu University Investigational Review Board approved this study. Patient informed consents were not obtained because of limitations such as patient death, break in contact, and follow-up occurring in other hospitals. All information about this study protocol that is described in the subsequent "Patients" section was opened by bulletin according to the Kyushu University Investigational Review Board guidelines. Patients could be notified about this study and could ask whether they were included in the study. All patients were automatically included in this study without consent; however, patients could request to be excluded.

Patients. We conducted a retrospective study of patients with CLI diagnosed as Fontaine III or IV peripheral artery disease (PAD) who underwent revascularization at the Department of Surgery and Science, Kyushu University Hospital, Fukuoka, Japan, between January 2002 and December 2009. CLI patients underwent imaging studies,

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Author conflict of interest: none.

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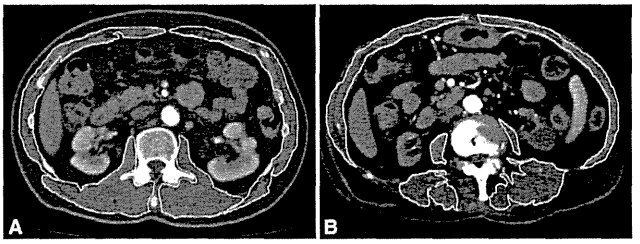

Fig 1. Representative transverse computed tomography (CT) images are shown of **(A)** a patient without sarcopenia and **(B)** a patient with sarcopenia. The images were taken at the third lumbar vertebra. The *white outline* shows the calculated skeletal muscle area.

including preoperative CT, angiography, and magnetic resonance angiography. PAD was categorized as Fontaine III or IV if the patient experienced pain at rest or had ulcers. Patients were excluded if they did not undergo CT because the quantity of their skeletal muscle could not be assessed.

Transverse CT images obtained at the third lumbar vertebra (L3) in the lower border were assessed in each patient. Skeletal muscles, including the psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal oblique abdominal muscle, and rectus abdominis muscle, were identified and quantified as previously described.[19,20,23] The cross-sectional areas (cm^2) of the skeletal muscles in the L3 region were measured by manual outlining on the CT images (Fig 1), and the areas were summed. The presence of sarcopenia was defined as a skeletal muscle area of <114.0 cm^2 for men and <89.8 cm^2 for women. These cutoff values were defined as below the fifth percentile of the standard value in healthy adults.[20,23] We also estimated the total skeletal muscle area using the method of Yoshizumi et al.[23]

The clinical characteristics, including smoking, diabetes mellitus, hypertension, dyslipidemia, ischemic heart diseases, cerebrovascular diseases, hemodialysis, and postoperative complications,[2-8] as well as survival time, were retrieved from the patients' medical records. We also developed a predictive score for sarcopenia based on patient characteristics. Overall survival (the primary end point) and clinical characteristics were compared between the two patient groups. Hazard ratios (HRs) for overall survival were calculated for each risk factor in univariate and multivariate analyses.

Follow-up strategy. All patients were examined in an outpatient clinic 1 month after revascularization, with subsequent assessments every 3 months if no problems developed. Some patients were seen in other hospitals. Patients with symptoms suggestive of vascular complications underwent additional medical examinations. Medical examinations consisted of an interview to assess symptoms, physical examinations, and measurement of the ankle-brachial pressure index. Patients with potential vascular complications also underwent CT and angiography as necessary.

Statistical analysis. The associations of continuous and categoric variables with clinically relevant outcome variables were assessed using the Student *t*-test and the Fisher exact test, respectively. The overall survival curves were analyzed using the Kaplan-Meier method and compared with the log-rank test. Univariate analysis of clinicopathologic factors and overall survival were performed using the log-rank test. Multivariate analyses of clinicopathologic factors and overall survival were performed using the Cox proportional hazards model. All analyses were performed using JMP 9.0 software (SAS Institute Inc, Cary, NC). Values of $P < .05$ were considered statistically significant.

RESULTS

Between January 2002 and December 2009, 108 patients with CLI underwent revascularization at the Department of Surgery and Sciences, Kyushu University Hospital, Fukuoka, Japan. The study excluded 44 patients (40.7%) because they did not undergo preoperative CT. Therefore, 64 patients (59.3%) with CLI underwent preoperative CT and were included in this study.

Of the 64 eligible patients, 28 (43.8%) had sarcopenia and 33 (56.2%) did not. The mean follow-up period was 3.5 ± 2.1 years in all 64 patients, 4.1 ± 2.2 years in patients without sarcopenia, and 2.7 ± 1.7 years in patients with sarcopenia. The clinicopathologic characteristics of patients with or without sarcopenia are summarized in Table I. There were significant differences in age, skeletal muscle area, estimated skeletal muscle area, body mass index (BMI), the proportions of smokers, patients with cerebrovascular disease, and patients who required hemodialysis in patients with and without sarcopenia (all $P < .05$). The other clinicopathologic characteristics and postoperative complications were not significantly different between patients with and without sarcopenia. The cancer-related mortality rate was significantly different between patients with and without sarcopenia ($P = .04$).

Patients with sarcopenia tended to be older, had a lower BMI and estimated skeletal muscle area, and a greater proportion had cerebrovascular diseases and required hemodialysis. Considering these results, we developed a predictive score for sarcopenia, which was calculated from the

Table I. Characteristics of patients with and without sarcopenia

Variable[a]	*Patients without sarcopenia (n = 36)*	*Patients with sarcopenia (n = 28)*	P
Age, years	69.2 ± 11.8	73.8 ± 9.6	.04[b]
Sex			.93[c]
Male	24 (66.7)	19 (67.9)	
Female	12 (33.3)	9 (32.1)	
Skeletal muscle area, cm^2	124.1 ± 19.3	91.0 ± 16.9	<.001[b]
Estimated, cm^2	136.1 ± 27.6	113.0 ± 24.7	<.001[b]
BMI, kg/m^2	23.1 ± 3.5	19.4 ± 2.3	<.001[b]
Serum albumin, g/dL	3.71 ± 0.58	3.85 ± 0.52	.30[b]
Fontaine/Rutherford			.94[c]
III/4	17 (47.2)	13 (46.4)	
IV/5, 6	19 (52.8)	15 (53.6)	
Smokers	21 (58.3)	8 (28.6)	.02[c]
Hypertension	21 (58.3)	15 (53.6)	.70[c]
Diabetes mellitus	18 (50.0)	12 (42.9)	.57[c]
Dyslipidemia	3 (8.3)	2 (7.1)	.86[c]
Ischemic heart disease	12 (33.3)	13 (46.4)	.29[c]
Cerebrovascular disease	5 (13.9)	11 (39.3)	.02[c]
Hemodialysis	5 (13.9)	10 (35.7)	.04[c]
Treatment			.41[c]
Endovascular therapy	6 (16.7)	7 (25.0)	
Bypass	30 (83.3)	21 (75.0)	
Graft type			
Vein	13 (43.3)	14 (66.7)	.10[c]
Prosthetic	15 (50.0)	6 (28.6)	.13[c]
Composite	2 (6.7)	1 (4.8)	.78[c]
Distal anastomosis			
Above knee	15 (50.0)	10 (47.6)	.87[c]
Below knee	15 (50.0)	11 (52.4)	.87[c]
Postoperative complications	10 (27.8)	9 (32.1)	.70[c]
Surgical site infection	2 (5.6)	2 (7.1)	.80[c]
Stroke	1 (2.8)	1 (3.8)	.86[c]
Pneumonia	0 (0)	1 (3.8)	.25[c]
Heart failure	2 (5.6)	2 (7.1)	.80[c]
Bleeding	1 (2.8)	1 (3.8)	.86[c]
Lymphorrhea	2 (5.6)	0 (0)	.21[c]
Others	2 (5.6)	2 (7.1)	.80[c]
Cause of death			
Ischemic heart disease	0 (0)	2 (14.3)	.27[c]
Acute heart failure	2 (25.0)	4 (28.6)	.86[c]
Cerebrovascular disease	1 (12.5)	1 (7.1)	.68[c]
Abdominal organ ischemia	0 (0)	1 (7.1)	.45[c]
Pneumonia	2 (25.0)	2 (14.3)	.53[c]
Acute respiratory failure	0 (0)	2 (14.3)	.27[c]
Sepsis	1 (12.5)	2 (14.3)	.91[c]
Cancer	2 (25.0)	0 (0)	.04[c]
Operative death	0 (0)	1 (3.6)	.25[c]

BMI, Body mass index.
[a]Values for continuous data are shown as means ± standard deviation, and categoric data are shown as number (%).
[b]Student *t*-test.
[c]Fisher exact test.

following conditions: age >65 years, BMI <22 kg/m^2, estimated skeletal muscle area below the fifth percentile of the value of healthy adults, the presence of cerebrovascular disease, and the requirement for hemodialysis. The score ranged from 0 to 5, and the prevalence of sarcopenia was 0% (0 of 12), 22.2% (4 of 18), 52.9 % (9 of 17), 87.5% (14 of 16), and 100% (1 of 1) for scores of 0 to 1, 2, 3, 4, and 5, respectively (Table II).

The overall survival curves of patients with and without sarcopenia are shown in Fig 2. The 5-year overall survival rates were 23.5% ± 0.18% for patients with sarcopenia and 77.5% ± 0.09% for patients without sarcopenia. The overall survival rate was significantly different between patients with and without sarcopenia ($P = .001$).

Table III reports the results of the univariate and multivariate analyses that were conducted to identify prognostic factors for overall survival. The univariate analyses showed that the presence of sarcopenia (HR, 4.24; 95% confidence interval [CI], 1.69-11.7; $P = .002$), hemodialysis (HR, 4.07; 95% CI, 1.68-9.75; $P = .002$), and postoperative complications (HR, 2.98; 95% CI, 1.23-7.10; $P = .02$) were significantly associated with overall survival. The

Table II. Distributions of the predicted scores for sarcopenia

Score	*0*	*1*	*2*	*3*	*4*	*5*
Patients without sarcopenia, No.	3	9	14	8	2	0
Patients with sarcopenia, No.	0	0	4	9	14	1
Prevalence rate, %	0.0	0.0	22.2	52.9	87.5	100.0

multivariate analysis showed these associations remained statistically significant for sarcopenia (HR, 3.22; 95% CI, 1.24-9.11; $P = .02$), hemodialysis (HR, 4.30; 95% CI 1.60-11.4; $P = .004$), and postoperative complications (HR, 5.02; 95% CI, 1.90-13.7; $P = .001$).

DISCUSSION

The prognosis of CLI patients is very poor.[8] Prior studies have shown that hemodialysis and postoperative complications are associated with poor prognosis of patients with PAD,[8-13] whereas the current study revealed that sarcopenia is also associated with poor prognosis of CLI patients.

Although the 5-year survival rate of CLI patients with sarcopenia was poor (23.5%), the survival rate of patients without sarcopenia was much higher (77.5%) and similar to that of patients with intermittent claudication reported in Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II).[8] Earlier studies demonstrated that hemodialysis is a critical risk factor for reduced survival in CLI patients,[23] which was also apparent in the present study (Table II). The BASIL study showed that bypass is an appropriate treatment for CLI patients with an expected survival of >2 years,[1] but the predictors of survival >2 years are still being discussed. The presence of sarcopenia and hemodialysis could possibly predict the prognosis of CLI patients. The current results suggest that patients without hemodialysis or sarcopenia could receive more aggressive treatments because they might be expected to survive longer, whereas patients with sarcopenia might require minimally invasive treatment because their survival is expected to be shorter.[1] For example, arterial bypass may be appropriate for patients without hemodialysis or sarcopenia because of the good long-term patency of this intervention.[1,24-26]

We believe that sarcopenia is a biomarker for overall debilitation and that measuring sarcopenia as an indication of the frailty of patients would be useful. Sarcopenia is associated with a variety of factors, including age, nutrition, comorbidities, and activities of daily living. A combination of factors, each with a small effect individually, could act together and result in sarcopenia.[27,28] To determine indications for surgical treatment, assessing the patient's general condition is important. Sarcopenia is associated with a poor general condition and could help the surgeon to select the appropriate treatment.

Age, BMI, the proportions of smokers, and patients with cerebrovascular disease were significantly different

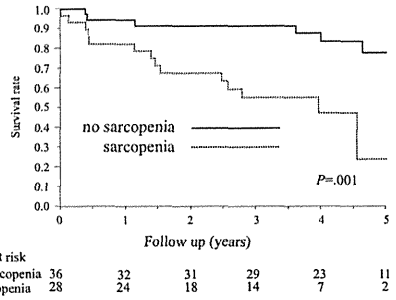

Fig 2. Kaplan-Meier curves show survival of patients with (*dotted line*) or without (*solid line*) sarcopenia ($P = .001$ by log-rank test).

between patients with and without sarcopenia; unexpectedly, they were not prognostic factors in the current study (Table III) but could predict the presence of sarcopenia based on the predictive score developed in this study (Table II). Indeed, patients with high predicted scores, based on a combination of risk factors, were expected to have sarcopenia. These findings suggest that these factors have a very small influence on the patient's prognosis, even though sarcopenia is caused by such factors. Instead, it is possible that the accumulation of these risk factors contributes to the onset of sarcopenia rather than its prognosis. These results may also highlight the prognostic role of sarcopenia in CLI patients.

Obesity is a major risk factor, but the patients in our study generally had a low BMI. This might be related to the general characteristics of Japanese people, whereas the incidence of obesity is significantly higher in Western countries. "Sarcopenic obesity" is a syndrome characterized by low skeletal muscle area but high fat content. Patients with sarcopenic obesity have a high BMI, but their skeletal muscle area is quite low. Because the prevalence of sarcopenic obesity may be quite high in Western countries, measuring skeletal muscle area seems to be quite important, even in patients with a high BMI, considering the poor prognosis of patients with sarcopenic obesity.[29]

The cancer-related mortality rate was significantly higher in patients without sarcopenia than in those with sarcopenia. Although considering the causes of death is important, there was no clear tendency toward an increased incidence of a specific cause of death in patients with sarcopenia in this current study. Therefore, further studies may be needed to assess the most common causes of death in patients with sarcopenia.

Sarcopenia is easy to diagnose by CT, and CT can also provide other useful anatomic information. Some patients were diagnosed by angiography, and endovascular interventions could be performed at the same time without CT scans. The current study revealed that patients without sarcopenia had a good prognosis. Arterial bypass may be

Table III. Univariate and multivariate analyses of clinicopathological factors associated with overall survival after treatment of critical limb ischemia (CLI)

	Univariate analysis		*Multivariate analysis*	
Variable	*HR (95% CI)*	P[a]	*HR (95% CI)*	P[b]
Age, years	4.98 (0.65-48.9)	.13		
Sex, male/female	2.53 (0.94-8.77)	.06		
Fontaine, IV/III	1.05 (0.45-2.50)	.91		
Serum albumin, g/dL	0.77 (0.35-1.68)	.50		
BMI, kg/m^2	0.52 (0.06-4.09)	.54		
Presence/absence of				
Sarcopenia	4.24 (1.69-11.7)	.002	3.22 (1.24-9.11)	.02
Smoking	0.68 (0.26-1.62)	.39		
Hypertension	1.03 (0.43-2.53)	.95		
Diabetes mellitus	2.32 (0.95-6.21)	.07		
Dyslipidemia	1.20 (0.19-4.19)	.81		
Ischemic heart disease	1.97 (0.82-4.75)	.12		
Cerebrovascular disease	1.86 (0.70-4.49)	.20		
Hemodialysis	4.07 (1.68-9.75)	.002	4.30 (1.60-11.4)	.004
Post-op complications	2.98 (1.23-7.10)	.02	5.02 (1.90-13.7)	.001
Treatment, bypass/EVT	2.33 (0.67-14.7)	.21		

BMI, Body mass index; *CI*, confidence interval; *HR*, hazard ratio; *EVT*, endovascular therapy.
[a]Log-rank test.
[b]Cox proportional hazards model.

suitable in these patients because it offers better long-term patency than endovascular interventions.[1,20,24,25] Therefore, preoperative CT should be considered in such cases, and the surgeon should select an appropriate treatment plan that takes into account the patient"s status, including sarcopenia.

Prior reports[19,20,23] have described methods to diagnose sarcopenia by CT, and these methods were used in this study. We thought that skeletal muscle area at the L3 level is suitable because this level includes multiple muscles involved in daily living activities. For example, the erector spinae is involved in maintaining an erect position, and the iliopsoas is used in walking. Cutoff values for skeletal muscle area have not been adequately defined. We used the fifth percentile of the value of healthy adults because it is widely recognized as a standard value statistically and was used in prior study.[20] We believe that establishing cutoff values of skeletal muscle area for predicting sarcopenia is important and that skeletal muscle area should be examined in various patient populations, including other races and age groups, worldwide.

Prior studies have discussed the mechanism by which sarcopenia may affect the prognosis of patients with some diseases,[27-31] but not CLI. Skeletal muscle was recently reported to be an endocrine organ.[15] Moreover, adiponectin[32] and carnitine,[33] which target skeletal muscle, were reported to improve arteriosclerosis. It was thought that sarcopenia could reduce the effects of adiponectin and carnitine, exacerbating whole-body arteriosclerosis, which might contribute to the poor prognosis of CLI patients, including those with arteriosclerosis. Considering these issues, improving the prognosis of patients with CLI may be possible by treating sarcopenia as a whole-body disease. In TASC II, treadmill training was recommended for intermittent claudication patients but not for CLI patients.[8] Resistance training and nutritional therapy have been reported to improve sarcopenia.[33] Further studies are needed to evaluate whether a comprehensive treatment program, including revascularization, resistance training, and nutrition therapy, improves the prognosis of CLI patients.

CONCLUSIONS

The results of this study imply that sarcopenia is a possible prognostic factor for overall survival in patients with CLI. Exercise and nutritional interventions focusing on improving sarcopenia might be useful treatment options for CLI patients and are a topic for future research.

AUTHOR CONTRIBUTIONS

Conception and design: YuM, TM
Analysis and interpretation: YuM, ST
Data collection: YuM, YA
Writing the article: YuM, JO
Critical revision of the article: TM, KM, KS
Final approval of the article: YoM
Statistical analysis: YuM, TM
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Overall responsibility: YoM

REFERENCES

1. Adam DJ, Beard JD, Cleveland T, Bell J, Bradbury AW, Forbes JF, et al. Bypass versus angioplasty in severe ischaemia of the leg (BASIL): multicentre, randomised controlled trial. Lancet 2005;366:1925-34.
2. McPhee JT, Nguyen LL, Ho KJ, Ozaki CK, Conte MS, Belkin M. Risk prediction of 30-day readmission after infrainguinal bypass for critical limb ischemia. J Vasc Surg 2013;57:1481-8.

3. Arvela E, Söderström M, Korhonen M, Halmesmäki K, Albäck A, Lepäntalo M, et al. Finnvasc score and modified Prevent III score predict long term outcome after infrainguinal surgical and endovascular revascularization for critical limb ischemia. J Vasc Surg 2010;52:1218-25.
4. Hirsch A, Criqui M, Treat-Jacobson D, Regensteiner J, Creager M, Olin J, et al. Peripheral arterial disease detection, awareness, and treatment in primary care. JAMA 2001;286:1317-24.
5. Dormandy JA, Murray GD. The fate of the claudicant—a prospective study of 1969 claudicants. Eur J Vasc Surg 1991;5:131-3.
6. Criqui M, Langer R, Fronek A, Feigelson H, Klauber M, Mccann T, et al. Mortality over a period of 10 years in patients with peripheral arterial disease. N Engl J Med 1992;326:381-6.
7. Fowkes F, Lee A, Murray G; on behalf of the ABI collaboration. Ankle-brachial index as an independent indicator of mortality in fifteen international population cohort studies. Circulation 2005;112:3704.
8. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, et al. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). Eur J Vasc Endovasc Surg 2007;33:S1-75.
9. Hoshino J, Fujimoto Y, Naruse Y, Hasegawa E, Suwabe T, Sawa N, et al. Characteristics of revascularization treatment for arteriosclerosis obliterans in patients with and without hemodialysis. Circ J 2010;74:2426-33.
10. Kimura H, Miyata T, Sato O, Furuya T, Iyori K, Shigematsu H. *Infrainguinal arterial reconstruction for limb salvage in patients with* end-stage renal disease. Eur J Vasc Endovasc Surg 2003;25:29-34.
11. Hakaim AG, Gordon JK, Scott TE. Early outcome of in situ femorotibial reconstruction among patients with diabetes alone versus diabetes and end-stage renal failure. J Vasc Surg 2003;27:29-34.
12. Ramdev P, Rayan SS, Sheahan M, Hamdan AD, Logerfo FW, Akbari CM, et al. A decade of experience with infrainguinal revascularization in a dialysis-dependent patient population. J Vasc Surg 2002;36:969-74.
13. Meyerson SL, Skelly CL, Curi MA, Desai TR, Katz D, Bassiouny HS, et al. Long-term results justify autogenous infrainguinal bypass grafting in patients with end-stage renal failure. J Vasc Surg 2001;34:27-33.
14. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. Age Ageing 2010;39:412-23.
15. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol 2012;8:457-65.
16. Montano-Loza AJ, Meza-Junco J, Prado CM, Lieffers JR, Baracos VE, Bain VG, et al. Muscle wasting is associated with mortality in patients with cirrhosis. Clin Gastroenterol Hepatol 2012;10:166-73.
17. Englesbe MJ, Patel SP, He K, Lynch RJ, Schaubel DE, Harbaugh C, et al. Sarcopenia and mortality after liver transplantation. J Am Coll Surg 2010;211:271-8.
18. van Vledder MG, Levolger S, Ayez N, Verhoef C, Tran TC, Ijzermans JN. Body composition and outcome in patients undergoing resection of colorectal liver metastases. Br J Surg 2012;99:550-7.
19. Harimoto N, Shirabe K, Yamashita YI, Ikegami T, Yoshizumi T, Soejima Y, et al. Sarcopenia as a predictor of prognosis in patients following hepatectomy for hepatocellular carcinoma. Br J Surg 2013;100:1523-30.
20. Masuda T, Shirabe K, Ikegami T, Harimoto N, Yoshizumi T, Soejima Y, et al. Sarcopenia is a prognostic factor in living donor liver transplantation. Liver Transpl 2014;20:401-7.
21. Antoun S, Borget I, Lanoy E. Impact of sarcopenia on the prognosis and treatment toxicities in patients diagnosed with cancer. Curr Opin Support Palliat Care 2013;7:383-9.
22. Shigematsu H, Nishibe T, Obitsu Y, Matsuzaki K, Ishida A, Miyata T, et al. Three year cardiovascular events and disease progress in patients with peripheral arterial disease: results from the Japan Medication Therapy for Peripheral Arterial Disease (J-METHOD). Int Angiol 2010;29:2-13.
23. Yoshizumi T, Shirabe K, Nakagawara H, Ikegami T, Harimoto N, Toshima T, et al. Skeletal muscle area correlates with body surface area *in healthy adults. Hepatol Res 2014;44:313-8.*
24. Wolfle KD, Bruijnen H, Loeprecht H, Rümenapf G, Schweiger H, Grabitz K, et al. Graft patency and clinical outcome of femorodistal arterial reconstruction in diabetic and non-diabetic patients: results of a multi-centre comparative analysis. Eur J Vasc Endovasc Surg 2003;25:229-34.
25. van der Zaag ES, Legemate DA, Prins MH, Reekers JA, Jacobs MJ. Angioplasty or bypass for superficial femoral artery disease? A randomized controlled trial. *Eur J Vasc Endovasc Surg 2004;28:132-7.*
26. Alber M, Romiti M, Brochado-Neto FC, Pereira CA. Meta-analysis of alternate autologous vein bypass grafts to infrapopliteal arteries. J Vasc Surg 2005;42:449-55.
27. Muscaritoli M, Anker SD, Argilés J, Aversa Z, Bauer JM, Biolo G, et al. Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics." Clin *Nutr 2010;29:154-9.*
28. Bauer JM, Sieber CC. Sarcopenia and frailty: a clinician's controversial point of view. Exp Gerontol 2008;43:674-8.
29. Vesser M, van Venrooij LM, Vulperhorst L, de Vos R, Wisselink W, van Leeuwen PA, et al. Sarcopenic obesity is associated with adverse clinical outcome after cardiac surgery. Nutr Metab Cardiovasc Dis 2013;23:511-8.
30. Genton L, Karsegard VL, Chevalley T, Kossovsky MP, Darmon P, Pichard C. Body composition changes over 9 years in healthy elderly subjects and impact of physical activity. Clin Nutr 2011;30:436-42.
31. Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N, Hioki K, et al. Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. J Biol Chem 2003;278:2461-8.
32. Brevetti G, Angelini C, Rosa M, Carrozzo R, Perna S, Corsi M, et al. Muscle carnitine deficiency in patients with severe peripheral vascular disease. Circulation 1991;84:1490-5.
33. Sakuma K, Yamaguchi A. Molecular mechanisms in aging and current strategies to counteract sarcopenia. Curr Aging Sci 2010;3:90-101.

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ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

Surgical Outcomes of Hepatic Resection for Hepatitis B Virus Surface Antigen-Negative and Hepatitis C Virus Antibody-Negative Hepatocellular Carcinoma

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ABSTRACT

Background. The incidence of hepatitis B virus surface antigen-negative and hepatitis C virus antibody-negative hepatocellular carcinoma (NBNC-HCC) is gradually increasing.

Methods. A retrospective cohort study was performed in 694 patients who underwent curative hepatic resection for primary HCC from January 1990 to December 2011.

Results. In the NBNC-HCC group ($n = 110$), the complication rate of diabetic mellitus (38 %) was significantly higher than that of the B-HCC group ($n = 110$; 17 %), and their rate of alcohol abuse (38 %) was significantly higher than that of both the B-HCC (26 %) and C-HCC groups ($n = 474$; 22 %). In the NBNC-HCC group, the tumor diameter (4.5 ± 3.6 cm) was significantly larger than that of the C-HCC group (2.9 ± 1.8 cm), but the rate of histological cirrhosis (37 %) was significantly lower than those of both the B-HCC (67 %) and C-HCC (53 %) groups. There were no significant differences regarding overall and disease-free survival among the three groups. In the NBNC-HCC group, multiple intrahepatic or distant recurrences (25 %) were significantly higher than in the C-HCC group (17 %), and the rate of recurrence more than 2 years after hepatic resection (24 %) was significantly higher than that of the B-HCC group (12 %).

Conclusions. The surgical outcomes of patients with NBNC-HCC were not significantly different compared with those of the patients with B-HCC or C-HCC. There was a substantial population with late recurrence among the patients with NBNC-HCC after curative hepatic resection, and thus not only long-term follow-up but also the early establishment of preventive methods for HCC recurrence from NBNC-hepatitis are necessary.

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide, accounting for approximately 6 % of all human cancers.[1] Infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is associated with approximately 85 % of HCC cases.[2,3] Today, more than 90 % of countries worldwide have introduced the HBV vaccine into their national infant immunization schedules, thus dramatically decreasing the incidence of HBV-related HCC.[4] For areas in which HCV infection is prevalent, such as Japan, the prevalence rates of cirrhosis and HCC are decreasing due to the development of antivirus therapy using interferon, whereas the prevalence HBV surface antigen (HBsAg)-negative/HCV antibody (HCVAb)-negative HCC (NBNC-HCC) is gradually increasing beyond 10 %.[2,5] In low-risk areas for HCV infection, including western countries, the rates of HCCs derived from alcohol-related liver disease (ALD), diabetes mellitus, or nonalchoholic steatohepatitis (NASH) associated with obesity have been reported to be increased.[6]

The different etiologies of HCC may cause different clinical characteristics and outcomes, thereby requiring different therapeutic strategies. In the past few decades, HBs-Ag-positive HCC (B-HCC) and HCVAb-positive HCC (C-HCC) have been thoroughly investigated.[7,8] The prognosis of C-HCC patients is worse than that of B-HCC patients, because multicentric carcinogenesis is more

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common in patients with C-HCC.[8] However, limited data regarding NBNC-HCC are available. According to the few reports concerning surgical results for patients with NBNC-HCC, the overall and disease-free survival rate are significantly better compared with those of C-HCC patients and similar to those of B-HCC patients.[9–12] However, several issues remain regarding the clinicopathologic features and biological behaviors of NBNC-HCC.

We present a retrospective analysis of the patient characteristics, operative results, and prognoses of individuals with primary NBNC-HCC compared with those with primary B-HCC or C-HCC.

METHODS

Patients' Characteristics

We retrospectively analyzed the cases of 712 patients with HCC who underwent curative hepatic resections at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, from January 1990 to December 2011. The 18 cases of patients who were both HBsAg- and HCVAb-positive were excluded from this analysis. We divided the remaining cohort of 694 patients into three groups; the B-HCC group ($n = 110$), the C-HCC group ($n = 474$), and the NBNC-HCC group ($n = 110$).

Surgical Procedures and Outcomes

The details of our surgical techniques and patient selection criteria for hepatic resection for HCC have been reported.[13,14] The resection volume was decided based on the patients' indocyanine green dye retention rate at 15 min (ICGR-15). Patients with an ICGR-15 $\geq$ 35 % were generally selected for limited resection.[14] Liver function criteria "Liver Damage (A, B, and C)" as proposed by the Liver Cancer Study of Japan was used in this study.[14]

In almost all hepatic resections, the Pringle's maneuver consisting of clamping the portal triad for 15 min and then releasing the clamp for 5-min intervals was applied; alternatively hemivascular occlusion was performed.[15] The CUSA system (Valleylab, Boulder, CO) was used to transect the liver parenchyma.

Any death that occurred in the hospital after hepatic resection was recorded as mortality. Complications were evaluated by Clavien's classification of surgical complications, and the complications with a score of Grade II or more were defined as positive.[16]

Follow-Up and Treatment Strategy for Recurrent HCC

After discharge, all patients were examined for recurrence by ultrasonography and tumor markers, such as α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), every month and by dynamic computed tomography every 3 months.[14] The median follow-up period after hepatic resection was 5.2 (range 1.1-21.4) years. When recurrence was suspected, we treated recurrent HCC by repeat hepatic resection at any times of recurrence, with ablation therapy, or by liodolization.[17,18]

Statistics

Continuous variables are expressed as the mean ± standard deviation (S.D.) and were compared using the Student's *t* test. Categorical variables were compared using the χ^2 test. The survival curves were generated by the Kaplan–Meier method and compared using the log-rank test. All analyses were performed with JMP® Pro 9.0.2 (SAS Institute Inc., Cary, NC). p values <0.05 were considered significant.

RESULTS

Patients' Background Characteristics

The patients' background characteristics are summarized in Table 1. The mean age in the NBNC-HCC group (66 ± 10 years) was significantly higher than that in the B-HCC group (55 ± 11 years; $p < 0.0001$). The complication rate of diabetes mellitus in the NBNC-HCC group (38 %) was significantly higher than that in the B-HCC group (17 %; $p = 0.0005$). The complication rate of daily drinking in the NBNC-HCC group (37 %) was significantly higher than those in both the B-HCC group (26 %; $p = 0.0322$) and the C-HCC group (22 %; $p = 0.0018$). The serum level of albumin in the NBNC-HCC group (4.0 ± 0.4 g/dL) was significantly higher than that in the C-HCC group (3.8 ± 0.2 g/dL; $p = 0.0011$). The serum levels of both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the NBNC-HCC group (43 ± 29 and 41 ± 30 IU/L, respectively) were significantly lower than those in the C-HCC group (57 ± 38 IU/L; $p = 0.0003$, and 57 ± 39 IU/L; $p < 0.0001$, respectively). The ICGR-15 value in the NBNC-group (16.2 ± 10.1 %) was significantly lower than that in the C-HCC group (19.7 ± 10.8 %; $p = 0.0025$).

Short-Term Surgical Outcomes

The patients' short-term surgical outcomes are summarized in Table 2. The mean resected volume in the NBNC-HCC group (247 ± 314 g) was significantly larger than that in the C-HCC group (116 ± 170 g; $p < 0.0001$). The performance rate of anatomical resection in the NBNC-HCC group (50 %) was significantly higher than that in the