For the evaluation of central muscle loss, preoperative computed tomography scans were used to measure the psoas muscle area, which is a valid part of a sarcopenia evaluation. In many reports, this has been complicated by the fact that specific area-tracing software or manual tracing was needed to calculate the psoas muscle area. In the current study, to simplify the measurements, the lengths of the major and minor axes of the psoas muscle were measured. The area of the psoas muscle was simply approximated with the radii of the major and minor axes.

It has been reported that enteral nutrition prevents intestinal mucosal atrophy and preserves intestinal structure and functions.[7] Previously, we have reported the beneficial impact of early enteral nutrition within the first 48 hours after LDLT in reducing postoperative sepsis.[8] However, the actual impact of early enteral nutrition on patients with sarcopenia is not known. Our second hypothesis is that there are some differences in the impact of early enteral nutrition on patients with sarcopenia and patients without sarcopenia.

The aims of this study were (1) to investigate sarcopenia as a novel predictor of mortality and sepsis after LDLT and (2) to evaluate the effects of early enteral nutrition on patients with sarcopenia.

PATIENTS AND METHODS

Patients

Two hundred twenty-eight recipients of LDLT performed at Kyushu University Hospital between November 2003 and December 2011 were retrospectively investigated. Twenty-three patients with acute hepatic failure and 1 patient who died from operative blood loss were excluded from this study. Psoas muscle measurements from computed tomography were available for 204 recipients. Written informed consent was obtained from all patients. The institutional review board approved this study.

Assessment of the Area of the Psoas Muscle

All study patients underwent preoperative computed tomography within the month before LDLT. Instead of using any area-measuring software, we simply measured the lengths of the major and minor axes of the psoas muscle at the caudal end of the third lumbar vertebra. The area of the psoas muscle was calculated with the following formula:

$$\text{Area} = a \times b \times \pi \quad (1)$$

where a and b are the radii of the major and minor axes, respectively.

In this study, for the definition of sarcopenia, we consulted our previous study of the cross-sectional area of the psoas muscle at the caudal end of the third lumbar vertebra of healthy donors.[9] An area of the psoas muscle lower than the 5th percentile for each sex was defined as sarcopenia. The cutoff levels were defined as 800 cm^2 for men and 380 cm^2 for women.[9]

Evaluation of the Prognostic Factors After LDLT

Predictors of sarcopenia were evaluated only with preoperative values. The following were used as preoperative factors: recipient age, donor age, recipient sex, recipient status, preoperative renal failure, body mass index (BMI), Child-Pugh class, Model for End-Stage Liver Disease (MELD) score, and graft volume/standard liver volume (GV/SLV) ratio. Prognostic factors were investigated with the foregoing preoperative values and sarcopenia.

Evaluation of the Correlation Between Sarcopenia and Postoperative Sepsis

Postoperative sepsis was defined as the isolation of bacteria other than common skin contaminants from a single blood culture within the first 3 months after transplantation along with clinical symptoms.[8,10] Risk factors for postoperative sepsis were investigated with the preoperative factors.

We introduced early enteral nutrition after LDLT in 2003. Initially, the adoption of enteral nutrition was determined on a case-by-case basis. Since 2008, early enteral nutrition via a nasojejunal tube has been routinely applied for all recipients within the first 24 hours after LDLT.[8] In order to evaluate the effects of early enteral nutrition on postoperative sepsis in patients with sarcopenia, the postoperative sepsis rates for patients with sarcopenia and patients without sarcopenia before and since 2008 were investigated.

Statistical Analysis

All values are expressed as means and standard deviations. Univariate analyses were performed with the chi-square test or Fisher's exact probability test for categorical values and with the Mann-Whitney U test for continuous variables. Overall survival rates were calculated and compared with the Kaplan-Meier method and the log-rank test or Cox regression. Multivariate analyses were performed with the Cox proportional hazards regression model for overall survival. Differences with a P value < 0.05 were considered to be significant. All statistical analyses were performed with StatView 5.0 (SAS Institute, Cary, NC).

RESULTS

Definition of Sarcopenia

The median calculated area of the psoas muscle was 530.6 cm^2 for all patients (range = 122.5-1667.5 cm^2), 760.9 cm^2 for male patients (range = 192.7-1667.5 cm^2), and 423.1 cm^2 for female patients (range = 122.5-1195.6 cm^2). Histograms of the area of the psoas muscle for all patients (Fig. 1A), male patients (Fig. 1B), and female patients (Fig. 1C) are shown. The histograms of all populations were normally distributed.

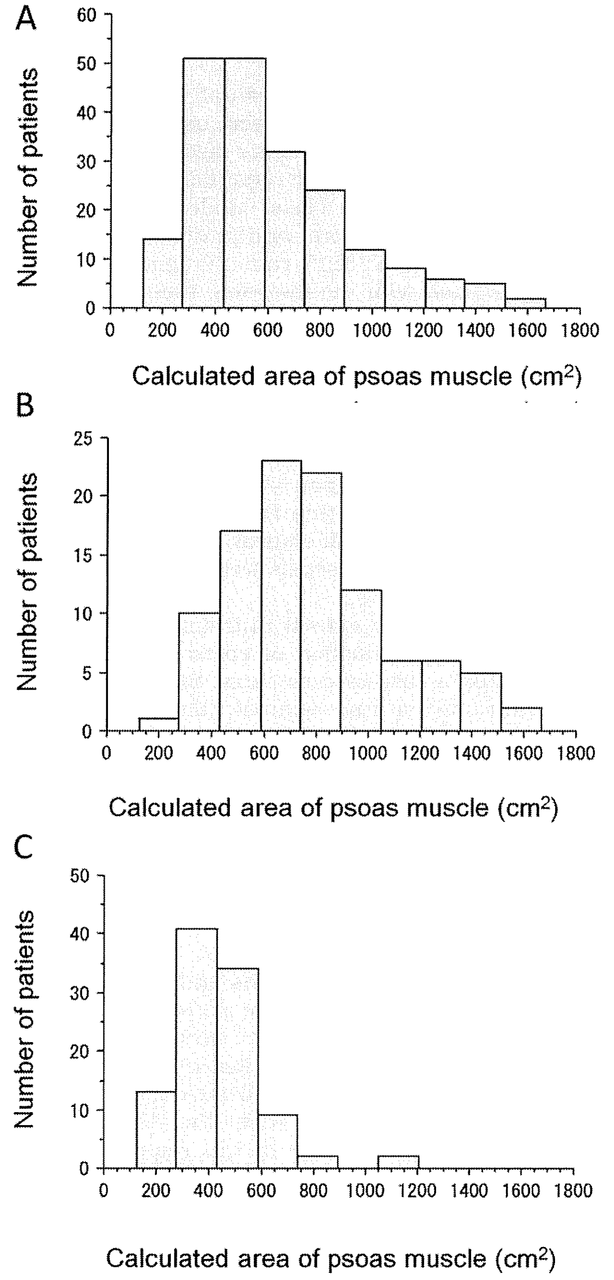

Figure 1. Histograms of the area of the psoas muscle for (A) all patients, (B) male patients, and (C) female patients. The histograms of all populations were normally distributed.

When we defined the cutoff levels as 800 cm^2 for men and 380 cm^2 for women on the basis of our previous data for healthy donors, 96 of the 204 patients (47.1%), including 58.3% (60/103) of the male patients and 35.6% (36/101) of the female patients, were diagnosed with sarcopenia.

Comparisons of the clinical characteristics of patients with sarcopenia and patients without sarcopenia are shown in Table 1. In the univariate analysis, the rates were higher in the sarcopenia group for the following variables: male sex (P = 0.001), hospitalized (P = 0.005), renal failure (P = 0.04), Child-Pugh class C (P = 0.02), and a MELD score ≥ 20 (P = 0.01). Patients with sarcopenia had lower BMIs (P = 0.004) than patients without sarcopenia. A logistic regression analysis revealed that a higher recipient age (P = 0.05), male sex ($P < 0.001$), and a lower recipient BMI (P = 0.002) were associated with sarcopenia.

As for the diagnoses of the recipients, 12 of 26 patients (46.2%) with hepatitis B virus–positive cirrhosis, 45 of 103 patients (43.7%) with hepatitis C virus–positive cirrhosis, 12 of 27 patients (44.4%) with primary biliary cirrhosis, 7 of 10 patients (70.0%) with alcoholic cirrhosis, and 20 of 38 patients (52.6%) with other diagnoses suffered from sarcopenia (P = 0.79).

Prognostic Factors After LDLT

Patients with sarcopenia showed significantly worse overall survival in comparison with patients without sarcopenia (P = 0.02; Fig. 2). The 3- and 5-year overall survival rates were 74.5% and 69.7%, respectively, for patients with sarcopenia and 88.9% and 85.4%, respectively, for patients without sarcopenia (P = 0.02). Twenty-three patients with sarcopenia died during the follow-up period. The causes of death were postoperative sepsis for 26.1% (6/23), recurrence of hepatocellular carcinoma for 21.7% (5/23), postoperative bleeding for 13.0% (3/23), and other causes for 39.1% (9/23).

The univariate analysis showed that patients with a lower overall survival rate after LDLT correlated with higher rates of preoperative renal failure (P = 0.01) and sarcopenia (P = 0.02; Table 2). In the multivariate analysis, only sarcopenia (hazard ratio = 2.06, P = 0.047) was an independent prognostic factor. Age, BMI, Child-Pugh score, MELD score, and GV/SLV ratio did not influence overall survival after LDLT.

Sarcopenia and Postoperative Sepsis

Twenty-five of the 204 patients experienced postoperative sepsis. The rate of postoperative sepsis was 17.7% (17/96) for patients with sarcopenia and 7.4% (8/108) for patients without sarcopenia (P = 0.03). Risk factors for postoperative sepsis were investigated. In the univariate analysis, recipient age (P < 0.001), donor age (P = 0.046), recipient status (P = 0.03), preoperative renal failure (P = 0.01), a MELD score ≥ 20 (P = 0.04), and sarcopenia (P = 0.03) were significant. A logistic regression analysis revealed that a lower recipient age (P < 0.001), a higher BMI (P = 0.02), and sarcopenia (P = 0.009) were significant risk factors (Table 3).

The effects of early enteral nutrition on postoperative sepsis were investigated in patients with sarcopenia and patients without sarcopenia. Early enteral nutrition within the first 48 hours after LDLT was performed for 24.2% (24/99) in 2003-2007 and for 100% (105/105) in 2008-2011. The incidence of postoperative sepsis was 18.2% (18/99) in 2003-2007 and 6.7% (7/105) in 2008-2011 (P = 0.02). In the

TABLE 1. Comparison of the Clinical Characteristics of Patients With Sarcopenia and Patients Without Sarcopenia

Variable	No Sarcopenia (n = 108)	Sarcopenia (n = 96)	Univariate Analysis: *P* Value	Multivariate Analysis: Hazard Ratio	Multivariate Analysis: 95% Confidence Interval	Multivariate Analysis: *P* Value
Recipient age (years)*	53.9 ± 10.5	54.8 ± 8.5	0.48	1.03	1.00-1.07	0.05
Donor age (years)*	34.4 ± 9.8	35.2 ± 11.2	0.59	1.01	0.98-1.04	0.50
Recipient sex: male/female [% (n)]	39.8 (43)/60.2 (65)	62.5 (60)/37.5 (36)	0.001	3.34	1.75-6.41	<0.001
Recipient status: hospitalized/home [% (n)]	20.4 (22)/79.6 (86)	38.5 (37)/61.5 (59)	0.005	1.95	0.90-4.23	0.09
Preoperative renal failure: yes/no [% (n)]	2.8 (3)/97.2 (105)	10.4 (10)/89.6 (86)	0.04	2.02	0.44-9.23	0.37
Recipient BMI (kg/m^2)*	24.2 ± 3.6	22.8 ± 3.1	0.004	0.86	0.78-0.95	0.002
Child-Pugh class: A + B/C [% (n)]	38.9 (42)/61.1 (66)	24.0 (23)/76.0 (73)	0.02	1.42	0.68-2.97	0.35
MELD score: ≥20/<20 [% (n)]	10.2 (11)/89.8 (97)	24.0 (23)/76.0 (73)	0.01	2.46	0.95-6.37	0.06
GV/SLV ratio (%)*	40.7 ± 7.7	41.3 ± 8.5	0.62	0.99	0.96-1.03	0.72

*The data are presented as means and standard deviations.

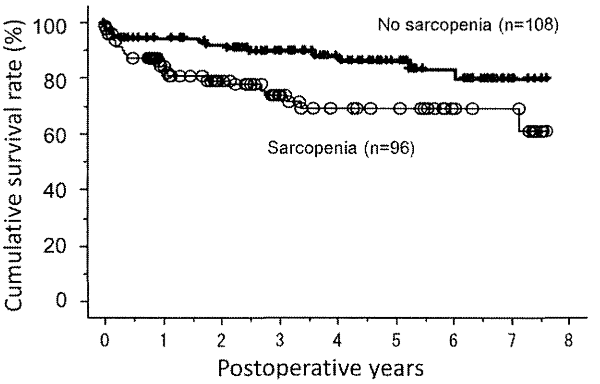

Figure 2. Overall survival and sarcopenia. Patients with sarcopenia had significantly worse overall survival than patients without sarcopenia (*P* = 0.02).

subgroup of patients without sarcopenia, the incidence of postoperative sepsis was 11.7% (7/60) in 2003-2007 and 2.1% (1/48) in 2008-2011 (*P* = 0.07). In the subgroup of patients with sarcopenia, the incidence of postoperative sepsis was 28.2% (11/39) in 2003-2007 and 10.5% (6/57) in 2008-2011 (*P* = 0.03; Table 4).

DISCUSSION

To determine sarcopenia, we measured the major and minor axes of the psoas muscle; we did not use any area-measuring software. Using such software is sometimes a little complicated; in particular, the tracing of the psoas muscle area may not always be correct. In the current study, the data were normally distributed well, and so they were considered to be reliable. There is no apparent definition of sarcopenia based on the psoas muscle area.[5] In many reports, the definition of sarcopenia has been decided subjectively on the basis of data from examinees.[5,11] In the current study, on the basis of data from our previous study, sarcopenia was defined as less than the 5th percentile value of the psoas muscle area of healthy donors of each sex.[9] The data for the psoas muscle area of the donors, both males and females, were also normally distributed,[9] so it was reasonable to define a cutoff value for patients with sarcopenia. Although an area less than the 5th percentile of the psoas muscle area of healthy donors was defined as sarcopenia, 58.3% of male recipients and 35.6% of female recipients were diagnosed with sarcopenia. Not surprisingly, more recipients than healthy donors had central muscle loss.

In this study, preoperative sarcopenia was an independent predictor of mortality after LDLT. Associations with sarcopenia and a poor prognosis have been reported not only for transplant patients[5,12] but also for cancer patients.[11,13] Sarcopenia seems to reflect a surgeon's clinical impression of disease severity. Actually, in the current study, the Kaplan-Meier curve for patients with sarcopenia was significantly lower than the curve for patients without sarcopenia in the early period after LDLT, and approximately 40% of the deaths were due to postoperative sepsis or bleeding.

It has been reported that approximately 40% of patients with cirrhosis suffer from sarcopenia.[4] Although the mechanism of sarcopenia in patients with cirrhosis has not been clarified, one of the most important causes is thought to be malnutrition. A poor nutritional status has been suggested to increase the risk of posttransplant complications or mortality.[14,15] Malnutrition has been reported in 60% to 80% of patients

TABLE 2. Univariate and Multivariate Analyses of the Impact of Sarcopenia and Other Clinical Characteristics on Overall Survival

		Univariate	Multivariate Analysis		
Variable	All Patients	Analysis: P Value	Hazard Ratio	95% Confidence Interval	P Value
Recipient age (years)*	54.4 ± 9.6	0.81	1.00	0.96-1.04	0.99
Donor age (years)*	34.8 ± 10.4	0.16	1.02	0.99-1.05	0.21
Recipient sex: male/female (n)	103/101	0.41	1.09	0.54-2.19	0.81
Recipient status: hospitalized/home (n)	59/145	0.37	1.00	0.44-2.28	0.99
Preoperative renal failure: yes/no (n)	13/191	0.01	2.60	0.78-8.62	0.12
BMI (kg/m^2)*	23.6 ± 3.4	0.18	1.09	0.98-1.20	0.10
Child-Pugh class: C/A + B (n)	139/65	0.39	1.10	0.48-2.56	0.81
MELD score: ≥20/<20	34/170	0.15	1.15	0.45-2.95	0.77
GV/SLV ratio (%)*	41.0 ± 8.1	0.80	0.99	0.95-1.03	0.63
Sarcopenia: yes/no (n)	96/108	0.02	2.06	1.01-4.20	0.047

*The data are presented as means and standard deviations.

TABLE 3. Univariate and Multivariate Analyses of Risk Factors for Postoperative Sepsis

		Univariate	Multivariate Analysis		
Variable	All Patients	Analysis: P Value	Hazard Ratio	95% Confidence Interval	P Value
Recipient age (years)*	54.4 ± 9.6	<0.001	0.88	0.83-0.94	<0.001
Donor age (years)*	34.8 ± 10.4	0.046	1.01	0.97-1.05	0.66
Recipient sex: male/female (n)	103/101	0.39	0.83	0.30-2.32	0.72
Recipient status: hospitalized/home (n)	59/145	0.03	2.20	0.70-6.91	0.18
Preoperative renal failure: yes/no (n)	13/191	0.01	2.45	0.49-12.2	0.28
BMI (kg/m^2)*	23.6 ± 3.4	0.66	1.19	1.03-1.38	0.02
Child-Pugh class: C/A + B (n)	139/65	0.82	0.43	0.12-1.61	0.21
MELD score: ≥20/<20	34/170	0.04	1.71	0.49-5.95	0.40
GV/SLV ratio (%)*	41.0 ± 8.1	0.23	1.05	0.99-1.12	0.13
Sarcopenia: yes/no (n)	96/108	0.03	5.31	1.53-18.4	0.009

*The data are presented as means and standard deviations.

TABLE 4. Incidence of Postoperative Sepsis

	Postoperative Sepsis [% (n/N)]		
	2003-2007 (n = 99)	2008-2011 (n = 105)	P Value
All patients (n = 204)	18.2 (18/99)	6.7 (7/105)	0.02
Patients without sarcopenia (n = 108)	11.7 (7/60)	2.1 (1/48)	0.07
Patients with sarcopenia (n = 96)	28.2 (11/39)	10.5 (6/57)	0.03

with cirrhosis. However, assessing the nutritional status of patients with liver dysfunction is difficult because of fluid collections caused by impaired protein synthesis in the liver.[16-18] The albumin and prealbumin levels do not necessarily reflect the nutritional status because hepatocellular protein synthesis is usually impaired in these patients. The assessment and interpretation of body weight are also difficult because of the presence of ascites, pleural effusion, and peripheral edema. Besides, sarcopenic, obese patients with respiratory and gastrointestinal tumors have recently been reported to have worse survival.[19] These facts may be the reasons that younger, high-BMI, and sarcopenic patients are at high risk for postoperative sepsis.

The incidence of postoperative sepsis was reduced even in patients with sarcopenia after the routine application of early enteral nutrition. However, the incidence was still high (10.5%). One of the reasons may be the lack of glutamine, especially in patients with sarcopenia. Glutamine is mainly synthesized in skeletal muscle, and that is reduced in sarcopenic patients.[20] Additionally, we used an enteral nutrition formula that does not include glutamine, and it is thought that patients with sarcopenia suffer from glutamine depletion. Glutamine is an important nutrient in constructing the intestinal wall: a decrease in glutamine can weaken the intestinal wall, and postoperative sepsis due to bacterial translocation may occur.[21] Besides, it has recently been reported that portal glucose delivery stimulated not liver but instead muscle protein synthesis in an in vivo study.[22] Protein synthesis in patients with sarcopenia must be lower than that in patients without sarcopenia. Now, a prospective study using early enteral nutrition with or without glutamine is being planned and promoted.

As for the benefits of this study, the most important difference between DDLT and LDLT may be the timing of liver transplantation. It can be easier to control the timing of the operation with LDLT. If a patient with sarcopenia is diagnosed in a candidate for LDLT, liver transplantation can be deferred, and previous treatments for sarcopenia (ie, nutritional and physical therapy) can be applied. The diagnosis of sarcopenia before transplantation can be more useful in LDLT versus DDLT. Branched-chain amino acids (BCAAs) are a source of energy, modulate signal transduction as messengers in skeletal muscle, and prevent muscle atrophy.[16,23,24] On the other hand, previous studies have shown the impact of changes in BCAA levels on the immune system. In vitro studies have shown that the omission of a single BCAA from a medium of cultured lymphocytes completely abolishes protein synthesis and cellular proliferation.[25-27] Kakazu et al.[28,29] demonstrated that an increased concentration of BCAAs could restore the functions of dendritic cells harvested from patients with cirrhosis both in vitro and ex vivo. Preoperative BCAA supplementation may have effects not only in preventing central muscle loss but also in restoring immune function in patients with advanced liver cirrhosis.

In conclusion, sarcopenia is an independent predictor of mortality and a risk factor for sepsis after LDLT. The incidence of postoperative sepsis was reduced even in patients with sarcopenia after the routine application of early enteral nutrition. Sarcopenia may be an objective evaluation of malnutrition in transplant candidates, and the treatment of malnutrition may improve mortality rates after liver transplantation. Further studies with larger numbers are required.

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ELSEVIER

Two-step Selection Criteria for Living Donor Liver Transplantation in Patients With Hepatocellular Carcinoma

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ABSTRACT

We have proposed risk factors for tumor recurrence, such as tumor nodule ≥5 cm and des-gamma-carboxy prothrombin ≥300 mAU/mL after living donor liver transplantation (LDLT) for hepatocellular carcinoma (HCC). The aim of this study was to clarify the risk factors for HCC recurrence and mortality within our criteria. We enrolled 152 adult recipients who had undergone LDLT for end-stage liver disease with HCC who met our criteria. The recurrence-free survival rates after LDLT were calculated. Risk factors for tumor recurrence were identified. On univariate analysis, factors affecting recurrence-free survival were pretransplant treatment for HCC, neutrophil-to-lumphocyte ratio (NLR) >4, alpha-fetoprotein ≥400 ng/mL, ≥5 nodules, and bilobar tumor distribution. Multivariate analysis identified that NLR >4 and ≥5 nodules were independent risk factors for tumor recurrence after LDLT (P = .003 and P = .002, respectively). Two-step selection criteria enable selection of patients who have high-risk of tumor recurrence.

HEPATOCELLULAR CARCINOMA (HCC) is the fifth most common neoplasm worldwide and the third most common cause of cancer-related death. Its incidence is increasing because of the dissemination of hepatitis B and C virus infection.[1] Liver transplantation (OLT), which offers the theoretical advantage of removing both the tumor and the organ that are at risk of developing future malignancy, is an established therapy for HCC in patients with liver cirrhosis.[2] In Asian countries, religious, living donor OLT (LDLT) is a choice for treating such HCC patients after various treatments, such as radiofrequency ablation (RFA), transarterial chemoembolization (TACE), and/or hepatic resection.[3] We have reported the outcome of LDLT for otherwise unresectable and/or untreatable HCC patients and have proposed 2 risk factors for recurrence-free survival: tumor size >5 cm and des-gamma-carboxy prothrombin (DCP) levels >300 mAU/mL [Kyushu University (KU) criteria].[4,5] More LDLTs for HCC patients have been performed under the KU criteria, thus generating a larger cohort.

The neutrophil-to-lymphocyte ratio (NLR) has recently emerged as a useful prognostic factor for recurrence of several gastroenterologic malignancies. NLR ≥5 has been reported to be a marker of survival in colorectal cancer patients.[6] Recently, it has been demonstrated that a preoperative NLR ≥5 is an adverse predictor of recurrence-free survival for patients undergoing hepatic resection for HCC.[7] Furthermore, an elevated NLR significantly increases the risk of HCC recurrence after OLT.[8,9]

These data have encouraged us to investigate whether NLR could be a risk factor for HCC recurrence, to create new selection criteria for HCC patients undergoing LDLT. The aim of the present study was to clarify the risk factors for HCC recurrence and mortality after LDLT in patients who met the KU criteria and to create new selection criteria.

PATIENTS AND METHODS

Patients

One hundred fifty-eight adult recipients underwent LDLT for end-stage liver disease with HCC at KU Hospital between April 1999 and December 2011. Six recipients did not meet the KU criteria.

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Therefore, 152 recipients were enrolled in the study. In 12 of 152 cases, LDLT was performed for indications other than HCC, and the 12 cases were included in this study as HCC was found on explant pathology. One hundred five recipients underwent pretransplant treatment for HCC, such as RFA, TACE, microwave coagulation therapy, and/or hepatic resection, depending on the recipient's liver function and tumor status. Graft types included left lobe with caudate lobe (LL+C) graft ($n = 95$), right lobe graft without the middle hepatic vein ($n = 52$), posterior segment graft ($n = 4$), and dual graft ($n = 1$). The etiology of liver cirrhosis was hepatitis C ($n = 109$), hepatitis B ($n = 27$), cryptogenic ($n = 6$), and alcohol abuse ($n = 5$), autoimmune hepatitis ($n = 3$), and primary biliary cirrhosis ($n = 2$). Our selection criteria to perform LDLT for HCC patients were as follows: (1) no modality except LDLT available to cure the patients with HCC; (2) no extrahepatic metastasis; and (3) no major vascular infiltration.[4,5] There were no restrictions on tumor size, number of nodules, or pretransplant treatment. Since we proposed the KU criteria, we have not performed LDLT for HCC patients who have both tumor size >5 cm and DCP level >300 mAU/mL.

Pretransplant imaging was used to estimate number of nodules, up-to-seven criteria, and Milan criteria. Alpha-fetoprotein (AFP) and NLR were measured just prior to LDLT.

Donor and Graft Selection

Donors were selected from candidates who hoped to be living donors.[10] Donors were required to be within the third degree of consanguinity with recipients or spouses and to be between 20 and 65 years of age. Eligible donors proceeded to the imaging studies, including chest and abdominal X-rays and 3-mm-slice computed tomography (CT) scans for graft volumetric analysis. Three-dimensional CT was introduced for volumetric analysis and delineation of vascular anatomy. The standard liver weight (SLW) of recipients was calculated according to the formula of Urata.[10] Graft weight (GW) was predicted by CT volumetric analysis. Our decision about graft type for recipients was based on the preoperatively predicted GW-to-SLW ratio. LL+C graft was used when the preoperatively predicted GW-to-SLW ratio was >35%. When GW-to-SLW ratio with LL+C graft was <35% and remnant donor liver volume after right lobectomy was >35%, right lobe graft was used. Posterior segment graft was considered when the donor's vascular anatomy was suitable to take a posterior segment.

Postoperative Management

Immunosuppression was initiated using a protocol based on either tacrolimus (Prograf; Astellas Pharma Inc, Tokyo, Japan) or cyclosporine (Neoral; Novartis Pharma K.K., Tokyo, Japan) with steroid and/or mycophenolate mofetil (MMF; Chugai Pharmaceutical Co Ltd, Tokyo, Japan). A target trough level of tacrolimus was set at 10 ng/mL for 3 months after LDLT, followed by 5 to 10 ng/mL thereafter. A target trough level of cyclosporine was set at 250 ng/mL for 3 months after LDLT, followed by 150 to 200 ng/mL thereafter. Methylprednisolone was initiated on the day of LDLT, then tapered and converted to prednisolone 7 days after LDLT. Prednisolone treatment was tapered and discontinued 6 months after LDLT. MMF was used in 134 recipients and was started at 1 g/d on the day after LDLT, then tapered and discontinued until 6 months after LDLT. A trough level was not measured for MMF.

All patients were followed monthly, and the median follow-up period was 1660 days, with 791 days and 2617 days as the 25th and 75th percentiles, respectively.

Table 1. Risk Factors for Tumor Recurrence: Univariate Analysis

Variables	*n*	Recurrence-free survival (%) 1 y	3 y	5 y	*P* value
Recipient variables					
Gender					
Male	85	91.2	89.8	87.5	.75
Female	67	98.4	88.3	88.3	
Age (y)					
>60	63	91.5	89.2	89.2	.88
≤60	89	96.4	89.9	87.7	
Etiology					
HCV	110	94.3	89.9	88.0	.90
Others	42	94.4	88.1	88.1	
Pretransplant MELD					
<15	119	94.6	88.5	86.9	.44
≥15	33	93.6	93.6	93.6	
Diabetes mellitus					
Yes	37	91.1	86.7	81.3	.43
No	115	95.4	90.2	90.2	
NLR					
>4	22	84.7	72.8	54.6	.0012
≤4	130	95.9	92.0	92.0	
Splenectomy					
Yes	94	93.1	88.5	88.5	.94
No	58	96.4	90.7	88.5	
Calcineurin inhibitor					
TAC	71	95.5	90.4	90.4	.82
CyA	78	93.3	88.6	86.5	
Donor variables					
Gender[a]					
Male	109	96.0	91.3	89.5	.13
Female	42	90.2	84.5	84.5	
Donor age (y)[a]					
>40	37	100	96.4	96.4	.10
≤40	114	92.6	87.2	85.6	
GW–SLW ratio					
<35	32	89.6	81.6	81.6	.19
≥35	120	95.6	91.4	89.8	
Tumor variables					
Pretransplant treatment for HCC					
Yes	105	91.7	84.4	82.6	.01
No	47	100	100	100	
AFP (ng/mL)					
≥400	25	82.9	71.9	62.9	<.0001
<400	127	96.6	92.8	92.8	
Bilobar tumor distribution					
Yes	69	89.0	81.8	79.4	.003
No	83	98.7	95.8	95.8	
Number of nodules					
≥5	38	77.5	63.4	63.4	<.0001
<5	114	100	97.8	96.0	

MELD, Model for End-stage Liver Disease; NLR, neutrophil-to-lymphocyte ratio; TAC, tacrotimus; CyA, cyclosporine; GW, graft weight; SLW, standard liver weight; AFP, alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin; LDLT, living donor liver transplantation; HCV, hepatitis C virus.
[a]A case that used dual graft was excluded.

Post-LDLT Tumor Recurrence and Risk Factors

All patients had abdominal CT scan every 3 months and had chest CT scan and bone scintigraphy every 6 months for 5 years after

Table 2. Risk Factors for Tumor Recurrence: Multivariate Analysis

Variables	Odds ratio	95% CI	*P* value
Number of nodules ≥5	10.3	2.04–77.7	.002
NLR >4	7.73	2.04–26.4	.003
Pretransplant treatment for HCC[a]	4.81	0.63–∞	.14
AFP ≥400 ng/mL	2.13	0.46–8.56	.39
Bilobar distribution	0.92	0.12–8.87	>.999

NLR, neutrophil-to-lymphocyte ratio; AFP, alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin; HCC, hepatocellular carcinoma.
[a]Median unbiased estimates.

LDLT. Tumor recurrence was defined when any imaging studies, such as chest or abdominal CT scan or bone scintigraphy, revealed recurrence of HCC. Recurrence-free survival was defined as the time between LDLT and tumor recurrence. Univariate and multivariate analyses were performed to identify the factors associated with the recurrence-free survival after the LDLT.

Statistical Analysis

Recurrence-free survival rates were calculated by the Kaplan–Meier product-limited method. Cox regression analysis was applied to the multivariate analyses. Variables that were used for the analysis included recipient age, donor age, Model for End-stage Liver Disease score, presence of hepatitis C virus, presence of diabetes mellitus, recipient sex, donor sex, GW-to-SLW ratio, pretransplant treatment for HCC, number of nodules obtained by imaging study, pretransplant NLR, pretransplant AFP, tumor distribution, splenectomy, and a type of calcineurin inhibitor. All statistical analyses were performed using JMP 9.0 software (SAS, Inc, Cary, NC, USA). A *P* value of <.05 was considered significant.

RESULTS

Fifty-seven of 152 recipients (38%) did not meet the Milan criteria. The 1-, 3-, and 5-year recurrence-free survival rates in the recipients were 94.4%, 89.4%, and 88.1%, respectively. Sixteen of the 152 recipients had HCC recurrence after LDLT. Fifteen of those recurrent recipients did not meet the Milan criteria but were within KU criteria. Univariate analysis revealed that pretransplant treatment for HCC, NLR >4, AFP ≥400 ng/mL, ≥5 nodules, and bilobar tumor distribution were risk factors for HCC recurrence after LDLT ($P = .01$, $P = .001$, $P < .0001$, $P < .0001$, and $P = .003$, respectively; Table 1). Multivariate analysis revealed that NLR >4 and ≥5 nodules were independent risk factors for tumor recurrence after LDLT ($P = .003$ and $P = .002$, respectively; Table 2). The enrolled 152 recipients were divided into 3 groups according to score for risk factors for HCC recurrence. The recipients in group 1 had no risk factor ($n = 97$). The recipients in group 2 had a sum of risk factors equal to 1 ($n = 50$). The recipients in group 3 had a sum of risk factors equal to 2 ($n = 5$). The 1-, 3-, and 5-year recurrence-free survival rates of recipients in group 1 were all 100%. The 1-, 3-, and 5-year recurrence-free survival rates in group 2 were 89.1%, 74.0%, and 69.4%, respectively. The 1-year recurrence-free survival rate in group 3 was 30.0%. The 3- and 5-year recurrence-free survival rates were not available. Duration of LDLT and recurrence was 74 days, 89 days, and 219 days, respectively. Twelve recipients in group 2 had HCC recurrences after LDLT. The mean duration of LDLT and recurrence was 571 days. The recurrence-free survival rates of recipients in group 3 were significantly worse than those of recipients in group 1 and group 2 ($P < .0001$; Fig 1).

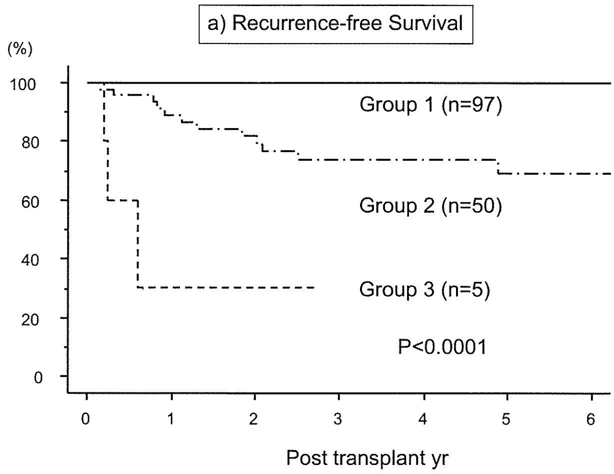

Fig 1. Recurrence-free recipient survival after living donor liver transplantation for hepatocellular corcinoma (HCC). The 152 recipients were divided into 3 groups according to score for risk factors for HCC recurrence. The 1-, 3-, and 5-year recurrence-free survival rates of recipients in group 1 ($n = 97$) were all 100%. The 1-, 3-, and 5-year recurrence-free survival rates in group 2 ($n = 50$) were 89.1%, 74.0%, and 69.4%, respectively. The 1-year recurrence-free survival rate in group 3 ($n = 5$) was 30.0%. The recurrence-free survival rates of recipients in group 3 were significantly worse than those of recipients in groups 1 and 2 ($P < .0001$).

DISCUSSION

It is crucial to exclude HCC patients with high risks of tumor recurrence. We should focus on how we can predict the

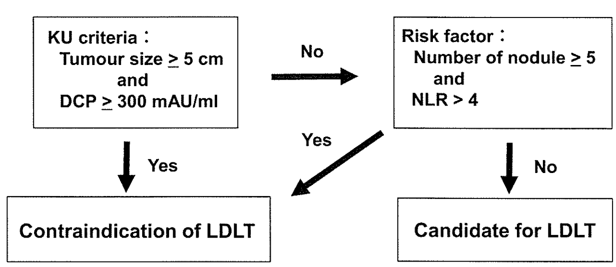

Fig 2. Two-step selection criteria to prevent hepatocellular corcinoma (HCC) recurrence after living donor liver transplantation (LDLT). When HCC patients have a maximum tumor size ≥5 cm and des-gamma carboxy prothrombin ≥300 mAU/mL (beyond the Kyushu University [KU] criteria), they are contraindicated for LDLT. Even when HCC patients meet the KU criteria, those with ≥5 nodules and neutrophil-to-lymphocyte ratio >4 are contraindicated for LDLT. The other HCC patients are good candidates for LDLT.

high-risk patients before LDLT. Therefore, for univariate and multivariate analysis, we chose variables that were obtained before transplantation. We observed that NLR >4 and ≥5 nodules were independent risk factors of HCC recurrence after LDLT for patients with HCC who met the KU criteria. The recipients were well stratified according to the number of risk factor.

By using receiver operating characteristics (ROC) analysis for tumor recurrence after LDLT, the area under the ROC curve of NLR was 0.695. A cutoff value of NLR was set as 4 using the analysis (data not shown). There are several possible mechanisms to explain the predictive role of preoperative elevated NLR.[11] Infiltration of proinflammatory macrophages, cytokines, and chemokines in the tumor microenvironment can boost tumor growth, invasion, and metastases.[12,13] Furthermore, high expressions of granulocyte colony-stimulating factor in tumor tissue and macrophage colony-stimulating factor in peritumoral tissue are associated with the elevated circulating neutrophils and poor prognosis.[14] However, reduced lymphocyte infiltration is a predictor of HCC recurrence after OLT.[15] The interpretation of NLR in patients with end-stage liver disease, often complicated with hypersplenism and pancytopenia, requires to need caution. Therefore, there may be limitation for the evaluation in such patients. Mean white blood cell (WBC) count of the patients was 3466/mm^3 in the present study (range 1060–8700). It was interesting that WBC count of patients with NLR >4 were higher than that of patients with NLR ≤4 ($P = .003$).

We will continue to use the KU criteria as the first exclusion criteria for LDLT. According to our results, we can use 2-step selection criteria for HCC patients as shown in Fig 2. In the first step, which is actually the same as the KU criteria that we used, patients are selected by tumor size and level of DCP. For patients who meet the KU criteria, patients are selected by NLR level and number of nodules.

In conclusion, our 2-step selection criteria enable selection of patients who have high risk of tumor recurrence. LDLT should not be performed in patients with HCC with NLR >4 and ≥5 nodules to achieve better outcome.

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TRANSPLANTATION

3D printing of the liver in living donor liver transplantation

Toru Ikegami and Yoshihiko Maehara

Advances in 3D printing techniques are gathering pace. With regard to living donor liver transplantation (LDLT), 3D printing could enable accurate assessment of liver volume and accurate visualization of liver anatomy, and could be particularly helpful for paediatric LDLT.

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Within the past few years, advances in 3D printing techniques have led to its introduction in medical fields. Zein *et al.*[1] have now used 3D printing in the setting of living donor liver transplantation (LDLT), by creating semi-transparent hepatic prototypes containing visible hepatic vessels and bile ducts, and a near-identical volume to the donor's liver.

Advances in multidetector CT and data processing techniques mean that virtual 3D imaging, virtual hepatectomy and virtual volumetry are now routinely performed during liver surgery.[2–4] However, because 3D images are visualized on a flat computer screen, surgeons are unable to manipulate the visualized liver with their hands. The development of 3D printing can overcome this limitation by enabling us to print and manipulate prototypic objects. Moreover, by carefully selecting the casting materials and dyes, it is possible to use different colours, transparencies, textures and consistencies to generate a prototype that mimics the real-life object.

For LDLT, it is essential to accurately predict the volume of a procured liver graft and to plan the resection route. If pre-transplant volumetry overestimates the volume of a procured liver, the recipient might develop small-for-size graft syndrome, which might result in graft loss—although graft quality is ultimately determined by multiple factors such as donor age, graft steatosis, disease severity and portal venous pressure.[5–7] Furthermore, if the resection route deviates from the planned route, it might lead to the procurement of a smaller-than-expected graft or increase the risk of donor complications caused by surgical injury to the donor's remnant liver. However, the liver is not transparent and the internal structures, especially the blood vessels and biliary tracts, are not visible. A 3D model that can be manipulated and that enables surgeons to visualize the internal structures could, therefore, overcome these issues.

> **“A 3D-printed model of the liver could help to reduce the risk of large-for-size syndrome...”**

The clinical application of 3D printing, as reported by Zein *et al.*,[1] has two main advantages, namely accurate assessment of the liver volume and accurate visualization of liver anatomy with easily visible structures. In terms of the accuracy of assessing the explanted liver volume, Zein *et al.*[1] reported that the 95% confidence interval was 28.8 ml (2.8% of the mean native liver volume), which suggests that the method is very accurate, especially compared with earlier reports of 3D virtual volumetry, for which the accuracy ranged from 5–25%.[2–4] The discrepancy between the pre-transplant expected liver volume and the actual volume or weight, even with accurate imaging and reconstruction, has been widely discussed. Factors that contribute to this discrepancy include conversion of intraoperative graft weight/volume at a rate of 1.0 g/ml, drainage of intrahepatic blood after procurement, dehydration of the perfused liver by hypertonic preservation solution, unevenness or deviation of the resection plane, and the loss of perfusion pressure after explanting the procured liver.[2–4] Clearly, accurately determining liver volume is difficult, even with volumetry. Zein *et al.*[1] determined actual graft volume, not weight, after procurement but before perfusion using a liquid displacement technique to minimize bias, enabling them to accurately measure the length, width, height and volume of the graft.

Although 3D printing techniques are suitable for adult-to-adult LDLT, they might be particularly useful in the setting of paediatric LDLT. In young children and small babies, one of the major obstacles to successful LDLT is large-for-size syndrome, in which the transplanted graft cannot be placed in a small abdominal cavity.[8] Large-for-size syndrome is associated with an increased risk of vascular complications including portal vein thrombosis, hepatic artery thrombosis and hepatic venous stenosis. Tissue oxygenation might also be impaired because of inappropriate compression.[8] To minimize these complications, surgical techniques have been developed to procure small grafts, including reduced lateral segment, mono-segment or reduced mono-segment grafts.[9] A graft-to-recipient weight ratio >4.0 is associated with increased risk of large-for-size syndrome, although a sculptured graft can be placed in the upper right abdominal cavity of a baby providing the vasculature is properly aligned.[8,9] A 3D-printed model of the liver could help to reduce the risk of large-for-size syndrome, especially if using reduced grafts to minimize tissue loss from the potential donor, and if the abdominal cavity of the recipient is also printed to test whether the planned graft fits the cavity.

> **“Another benefit of 3D printing is the ability to create a structure with visible interior structures”**

"...we believe that 3D printing is eminently suitable for LDLT"

Another benefit of 3D printing is the ability to create a structure with visible interior structures. As Zein *et al.*[1] showed in their figures, the portal and hepatic veins are visible from the cut surface of the prototype liver grafts. During hepatic resection, the surgeon exposes structures by dissecting opaque brown-coloured hepatic parenchyma and divides them when necessary. A deviation from the planned resection line of 1 cm might result in migration into a different segment, especially around the hepatic hilum. Thus, recognizing the structures along the resection line as well as the interior structures likely to be encountered during resection should fill the surgeon with confidence to perform a safe and secure hepatectomy. Indeed, 3D printing might be most useful when planning curvilinear hepatic resection, including subsegmentectomy of segments 7 or 8, because the right subphrenic dome portion of the liver is very deep and resection requires full mobilization of the right liver with the surgeon's right hand.[10] Such procedures are very difficult to visualize, even with 3D virtual simulation, unlike the flat cutting plane in hepatic lobectomy during donor surgery.

Some limitations of 3D printing in liver surgery include its high cost, the time needed (often >1 day) to generate the 3D prototypes, and the limited qualities and properties of the printing techniques and materials, which limit the transparency, flexibility and durability of the prototypes. Because of its high cost, 3D printing might not be justified for routine use in liver surgery. The long time to generate the 3D prototypes also limits the use of 3D printing to elective cases, preventing its use in acute cases. Nevertheless, w, and that further developments in 3D printing technologies, together with increasing availability, will lead to its wider application, enabling improvements in patient care, enhanced surgical education and the opening of new research fields.

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Competing interests
The authors declare no competing interests.

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RECTAL CANCER

Is 'watch and wait' a safe option for rectal cancer?

Bruce D. Minsky

The standard treatment for stage III rectal cancer is chemoradiation followed by radical surgery. Recent trials have recommended a 'watch and wait' approach for patients who achieve a complete clinical response. A new study reports that 51% of patients who achieved a sustained complete clinical response did not require radical surgery.

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The conventional adjuvant treatment for stage III rectal cancer is preoperative radiation with concurrent chemotherapy (chemoradiation) followed by radical surgery 4–8 weeks later. Radical surgery is not performed in some clinical settings. Historically, these settings have included early stage tumours, medically inoperable disease and patient refusal. In the past decade, not immediately treating patients with radical surgery has been used in patients who respond well to preoperative chemoradiation, which could enable some patients to avoid surgery altogether. A recent report by Habr-Gama *et al.*[1] is the fifth prospective trial examining this 'watch and wait' approach.

Although patients with rectal cancer can be cured without surgery, the results are often suboptimal. For example, Brierley and colleagues treated patients who refused surgery or had unresectable or medically inoperable disease with pelvic radiation alone and achieved a 5-year survival of 27%.[2] By contrast, preoperative chemoradiation followed by radical surgery results in 75% 5-year survival.[3]

Four prospective series have reported on chemoradiation followed by observation, in addition to the current study by Habr-Gama and co-workers (Table 1). An early series published in 2004 by Habr-Gama included 265 patients.[4] Overall, 27% achieved a complete clinical response (cCR) after chemoradiation and were selected for observation with close follow-up. Patients with stage cT1–3 disease were included and those who developed a local recurrence in the first year of follow-up were excluded from the analysis. Over a mean follow-up of 57 months, 3% of the patients had a luminal recurrence, 4% developed distant metastasis and 100% survival at 5 years was reported. In a subsequent update published in 2006, the local

"Selecting patients for a nonoperative approach on the basis of tumour response is reasonable"

Prognostic Factors Affecting Survival at Recurrence of Hepatocellular Carcinoma After Living-Donor Liver Transplantation: With Special Reference to Neutrophil/Lymphocyte Ratio

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Background. In living-donor liver transplantation (LDLT) for hepatocellular carcinoma (HCC), it is important to predict not only who may be susceptible to recurrence but also who may survive longer. The neutrophil/lymphocyte ratio (NLR) is useful to properly assess the patient without decreasing the long-term survival after LDLT. In this study, we investigated the relationship between NLR and prognosis of patients with recurrent HCC after LDLT.
Methods. In total, 167 LDLTs for HCC were enrolled in this study. Clinicopathologic factors for HCC recurrence after LDLT were investigated and prognostic factors were examined with respect to survival.
Results. The following factors were found to be significant in patients with HCC recurrence compared with the controls: α-fetoprotein ≧300 ng/mL, des-γ-carboxyprothrombin ≧300 mAU/mL, NLR ≧4, tumor number >3, tumor size ≧5 cm, duration of last treatment of HCC to LDLT <3 months, Milan criteria exceeded, histologic tumor number ≧10, histologic tumor size >5 cm, poor differentiation, presence of histologic vascular invasion, adjuvant chemotherapy, and interferon therapy against patients with hepatitis C virus. Male sex, interferon therapy against patients with hepatitis C virus, α-fetoprotein ≧300 ng/mL at recurrence, NLR ≧4 at recurrence, and nonsurgical resection for recurrent HCC were significantly related to poor prognosis. The 3-year survival rate after recurrence was 0% in patients with NLR ≧4 and 43.6% in patients with NLR <4. NLR was reelevated after LDLT in patients who later died; however, NLR gradually decreased in surviving patients.
Conclusion. NLR at recurrence is a prognostic factor affecting survival after recurrence in LDLT for HCC.

Keywords: Hepatocellular carcinoma, Living-donor liver transplantation, Recurrence, Neutrophil/lymphocyte ratio, Biomarker.

(*Transplantation* 2013;96: 1008–1012)

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world (*1, 2*). Because of advances in the diagnosis and management of HCC, significant improvements in the overall survival rate for HCC after hepatectomy have been achieved. However, even when curative resection is performed, the high postoperative recurrence rate remains an issue. Liver transplantation (LT) is currently the treatment of choice for early unresectable HCC owing to poor liver function and with candidate selection according to the Milan criteria (one nodule of 5 cm or two to three nodules all of 3 cm) (*3, 4*). Some LT centers have expanded the criteria, such as the up-to-seven criteria (*5*), because of the concern that the Milan criteria are too stringent. In Japan, some biomarkers, such as α-fetoprotein (AFP), des-γ-carboxyprothrombin (DCP), or neutrophil/lymphocyte ratio (NLR), in addition to the tumor size and the number of tumors, have been reported to be useful to properly assess the candidate without decreasing the long-term survival after living-donor LT (LDLT) (*6–8*).

A high NLR has been reported to be a predictor of poor survival after hepatic resection, radiofrequency ablation, transarterial chemoembolization, and LT for HCC. We recently showed that NLR was an important prognostic factor in patients with HCC after hepatic resection (*9*) and patients who underwent LDLT (*10*).

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Patients who experience recurrence after LT show rapid progression of recurrent disease and have a very poor prognosis because the rate of progression of recurrent HCC is more rapid after transplantation than after hepatic resection (*11, 12*). However, some patients have a good prognosis if they are appropriately treated after recurrence. Hence, it is important to predict not only who is likely to exhibit recurrence but also who may survive longer. There are no reports about the relationship between NLR and patients with recurrent HCC after LDLT, and there is little information regarding prognosis and treatment for HCC recurrence after LT. Therefore, in this study, we investigated the relationship between preoperative and postoperative NLR and prognosis of patients with recurrent HCC after LDLT.

RESULTS

In total, HCC recurrence was identified in 26 (15.5%) patients: 16 men and 10 women among the 167 patients with HCC. The mean duration until the initial recurrence after LDLT was 3.7 years, and the mean duration until death the initial recurrence was 1.7 years. Clinicopathologic factors on recurrence of HCC after LDLT using univariate analysis are shown in Table 1 and Table S1 (see **SDC**, http://links.lww.com/TP/A868). AFP ≧300 ng/mL, DCP ≧300 mAU/mL, NLR ≧4, tumor number >3, tumor size ≧5 cm, duration of last treatment of HCC to LDLT <3 months, Milan criteria exceeded, histologic tumor number ≧10, histologic tumor size >5 cm, poor differentiation, presence of histologic vascular invasion, adjuvant chemotherapy, and interferon (IFN) therapy against patients with hepatitis C virus (HCV) were significant differences between patients with recurrence and without recurrence of HCC. There were no significant differences regarding host-related factors except IFN between the two groups.

The prognostic factors for survival after recurrence using univariate analysis are shown in Table 2. These data included both factors before LDLT (Table 2) and those after LDLT (Table 3). Male sex, IFN therapy against patients with HCV, AFP ≧300 ng/mL at recurrence, NLR ≧4 at recurrence, and nonsurgical resection for recurrent HCC were significantly related to poor prognosis. The survival curves after recurrence for the patients with NLR ≧4 at recurrence are illustrated in Figure 1. The 3-year survival curves after recurrence were 0% in patients with NLR ≧4 and 43.6% in patients with NLR <4. The 3-year survival curves after recurrence were 50% in females and 9.5% in males, whereas the 3-year survival curves after recurrence were 53.3% in patients with IFN therapy against HCV and 0% in patients without IFN therapy. Furthermore, the 3-year survival curve after recurrence were 0% in patients with AFP ≧300 ng/mL at recurrence and 28.4% in patients with AFP <300 ng/mL. The 3-year survival curves after recurrence were 41.7% in patients with surgical resection for recurrent HCC and 0% in patients without surgical resection for recurrent HCC. Interestingly, AFP and NLR before LDLT, in particular, were not related to survival after recurrence of HCC. Multivariate analysis was not performed because of the small sample size.

NLR was reelevated after LDLT in patients who later died, whereas NLR gradually decreased in surviving patients (Fig. 2).

DISCUSSION

Using univariate analysis, our retrospective study indicated that male sex, IFN therapy for HCV, NLR and AFP at recurrence, and surgical resection for recurrent HCC were poor prognostic factors for survival after recurrence of HCC among patients with LDLT. We recently proposed new selection criteria for LDLT in patients with HCC (*7*). A multivariate analysis identified independent risk factors for post-LDLT tumor recurrence including tumor size, the presence of eight or more tumors, and an NLR of 4 or more. These criteria could effectively exclude patients with biologically

TABLE 1. Patients and tumor characteristics between patients with recurrence and without recurrence of HCC

Factors	Patients with recurrent HCC (n=26)	Patients without recurrent HCC (n=141)	*P*
AFP (ng/mL) <300/≧300	12/14	126/15	0.001
DCP (mAU/mL) <300/≧300	12/14	122/19	0.001
NLR <4/≧4	16/10	125/16	0.001
Number of tumors ≦3/>3	16/10	108/33	0.002
Tumor size (cm) ≦5/>5	6/20	139/2	0.001
Duration of last treatment to LDLT			
<3/≧3 months	16/10	127/14	0.001
Milan criteria, yes/No	7/19	98/43	0.001
Number of tumors (histologic)			
<10/≧10	13/13	114/27	0.002
Tumor size (cm) (histologic)			
≦5/>5	9/17	137/4	0.001
Tumor differentiation (histologic)			
Well+moderate/poor	16/10	111/30	0.001
Vascular invasion (histologic)			
Yes/no	18/8	40/101	0.001
IFN			
Yes/no	8/11	69/30	0.032

TABLE 2. Clinicopathologic factors on survival after recurrence of HCC using univariate analysis

Factors before LDLT	Patients	Survival at 3 years (%)	*P*
Gender			
Male	16	9.5	0.006
Female	10	50.0	
Age (years)			
≦57	12	13.0	0.943
>57	14	36.5	
Hepatitis			
HCV	19	20.2	0.489
Non-HCV	7	45.7	
Child-Pugh classification			
A+B	14	0	0.066
C	12	44.2	
MELD score			
<15	21	34.2	0.157
≧15	5	0	
AFP (ng/mL)			
<300	14	32.7	0.709
≧300	12	15.6	
DCP (mAU/mL)			
<300	14	35.0	0.185
≧300	12	16.7	
NLR			
<4	16	26.7	0.981
≧4	10	24.2	
Number of tumors			
≦3	10	33.3	0.613
>3	16	25.0	
Tumor size (cm)			
≦5	20	28.4	0.818
>5	6	16.7	
Duration of initial HCC to LDLT			
<1 year	7	16.2	0.509
≧1 year	15	28.6	
Duration of last treatment to LDLT			
<3 months	16	35.4	0.191
≧3 months	10	11.1	
Milan criteria			
Yes	7	60.0	0.481
No	19	21.1	
Graft vs. standard liver volume (%)			
<35	6	29.6	0.976
≧35	20	20.8	
Age of donor (year)			
≦30	11	15.0	0.926
>30	15	36.2	

aggressive tumors before LT, promoting an extremely low recurrence rate.

The rate of HCC recurrence after transplantation has ranged from 8% to 22.7% in different studies (*13–16*). Patients who experience recurrence after LT show rapid progression of recurrent disease and have a very poor prognosis such that median survival after recurrence ranged from 7 to 9 months because the rate of progression of recurrent HCC is more rapid after transplantation than after hepatic resection (*15, 17*). The main reason for this poor outcome is that the progression of the disease is usually fast because of the immunosuppressed state after transplantation. However, some patients have a good prognosis if they are appropriately treated after recurrence. In this study, NLR and AFP at recurrence are useful biomarkers to predict the prognosis after

TABLE 3. Clinicopathologic factors on survival after recurrence of HCC using univariate analysis

Factors after LDLT	Patients	Survival at 3 years (%)	*P*
Number of tumors (histologic)			
<10	13	42.9	0.102
≧10	13	10.0	
Tumor size (cm) (histologic)			
≦5	17	28.6	0.488
>5	9	22.2	
Tumor differentiation (histologic)			
Well+moderate	10	27.8	0.819
Poor	16	11.5	
Vascular invasion (histologic)			
Yes	18	19.7	0.446
No	8	38.1	
Adjuvant chemotherapy			
Yes	11	27.3	0.630
No	15	25.0	
CNI			
CyA	13	18.2	0.653
Tac	13	32.3	
Steroid use			
Yes	17	14.1	0.134
No	9	42.9	
IFN against HCV			
Yes	9	53.3	0.013
No	11	0	
AFP (ng/mL) at recurrence			
<300	23	28.4	0.001
≧300	3	0	
DCP (mAU/mL) at recurrence			
<300	21	31.4	0.120
≧300	5	0	
NLR at recurrence			
<4	17	43.6	0.006
≧4	9	0	
Initial site of recurrence			
Liver	4	33.3	0.986
Extraliver	22	22.6	
Duration of LDLT to recurrence (years)			
>1	12	40.0	0.097
≧1	14	12.2	
Surgical resection for recurrent HCC			
Yes	14	41.7	0.002
No	12	0	

recurrent HCC. This is the first report to discuss the relationship between NLR and the prognosis in patients with recurrent HCC after LDLT.

It is difficult to treat recurrences because these tumors tend to be involved in multiple organs, and if the tumor recurs in a single organ, it usually manifests multiple lesions. These findings suggest that the aggressiveness of the tumor and the effectiveness of the treatment for the recurrent lesion were important to survival after recurrence. If the recurrent disease progressed slowly and if the recurrent lesion was locally controllable, patient survival could be prolonged. Hence, it is important to predict not only who may live but also who can survive longer after recurrence.

Roayaie et al. (*15*) described that the surgical treatment of recurrence was independently associated with significantly longer survival. Furthermore, several articles suggested that surgical treatment of recurrent tumors after LT should be considered whenever possible (*14–16*). However, the indications for surgical resection of recurrent HCC are a solitary tumor or curative resection; thus, there is possibility that the patient whose recurrence had more malignant behavior (multiple recurrence or multisite recurrence) was eliminated as a candidate of surgical treatment. Interestingly, none of the primary tumor characteristics were associated with survival after HCC recurrence. There was no association of the survival after recurrence such as tumor size, number of tumors, tumor marker at pre-LT, histologic differentiation, or vascular invasion. Schlitt et al. (*16*) also reported that no primary tumor characteristics were associated with survival after HCC recurrence. These findings suggest that the malignant phenotype of the recurrent HCC might be quite different from that of the primary HCC. In our study, a univariate analysis showed that sex, IFN therapy for HCV, AFP ≧300 ng/mL at recurrence, NLR ≧4 at recurrence, and surgical resection were significant factors for recurrent HCC. Tumor growth in recurrent HCC is quicker after LT mainly because of the need for permanent immunosuppression (*17*). NLR and AFP at recurrence may reflect the biological malignant behavior.

The molecular mechanism associated with elevated NLR and the prognosis of patients with HCC is associated

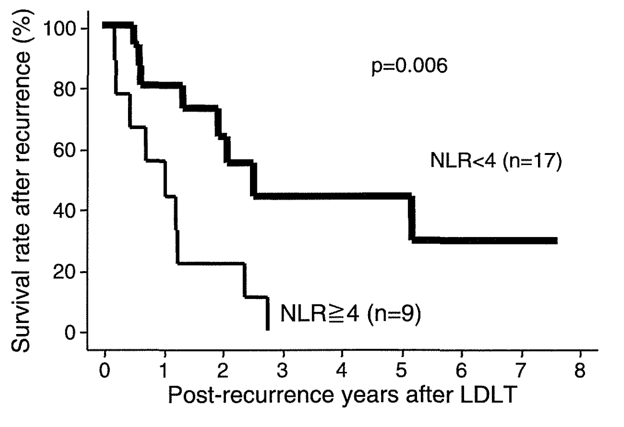

FIGURE 1. Survival after recurrence in patients with NLR<4 at recurrence or those with NLR≧4 at recurrence. Survival after recurrence in patients with NLR≧4 at recurrence was significantly poor prognosis.

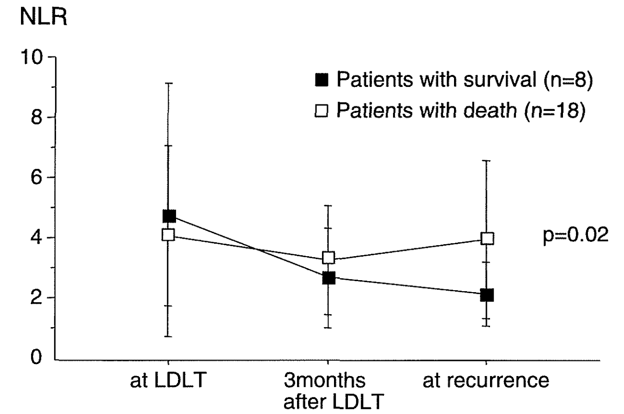

FIGURE 2. Time-dependent NLR at LDLT, 3 months after LDLT, and at recurrence. □, patients with death; ■, patients with survival. NLR was reelevated after LDLT in patients with death; on the contrary, NLR was gradually decreased with patients with survive.

with many factors, but it remains poorly understood. Chronic systemic inflammation is an important prognostic factor in patients with cancer. The NLR was used as a parameter of chronic inflammation in patients with cancer. We previously showed that NLR was an important prognostic factor in patients with HCC after hepatic resection (*9*) and in patients who underwent LDLT (*10*). A close relationship between accumulation of tumor-associated macrophages in HCC and high NLR levels was observed in patients with HCC who underwent hepatic resection and LDLT (*18*). A high NLR is associated with a high infiltration of tumor-associated macrophages and high inflammatory cytokine production in the tumor, such as interleukin-6 and interleukin-8, which promote systemic neutrophilia.

In conclusion, this retrospective analysis revealed that NLR at recurrence is a prognostic factor affecting survival after recurrence in LDLT for HCC. A multi-institutional study is needed to provide evidence of the significance of NLR in HCC.

MATERIALS AND METHODS

Patient Characteristics

A total of 393 LDLT operations were performed at Kyushu University Hospital from October 1996 to August 2012 after approval was obtained from the Ethics and Indications Committee of Kyushu University. Among them, 167 adult-to-adult LDLTs for HCC were enrolled in this study. The selection criteria for the HCC patients were as follows: (a) no modality, except LDLT available to cure patients with HCC and end-stage liver disease; (b) no extrahepatic metastasis; and (c) no major vascular infiltration, such as the portal vein or hepatic vein, thus indicating that there was no restriction on the tumor size or the number of the tumors.

The transplant procedures for both the donors and recipients have been described previously (6). The immunosuppressive regimen consisted of the combination of a calcineurin inhibitor (CNI) (tacrolimus [Tac] or cyclosporine A [CyA]) and steroid with or without mycophenolate mofetil. A steroid injection was given intravenously (methylprednisolone 1 g) and tapered to zero by day 7. Mycophenolate mofetil (1 g/day) treatment was started from postoperative day 1 and completed by 3 months. A maintenance immunosuppression therapy was conducted with low-dose Tac or CyA from postoperative day 7. Adjuvant systemic chemotherapy using 5-fluorouracil and cisplatin with or without gemcitabine for 1 month were administered to patients who had more than 300 mAU/mL DCP, more than 5 cm of maximum tumor size, or who exceeded the Milan criteria.

Prognostic Factor

The prognostic factors were examined with respect to survival after recurrence of HCC based on the following variables: sex (male vs. female), age (≧57 vs. <57 years), hepatitis (HCV vs. non-HCV), Child-Pugh classification (A+B vs. C), the Model for End-Stage Liver Disease (MELD) score (<15 vs. ≧15), serum AFP level (≧300 vs. <300 ng/mL), DCP level (≧300 vs. <300 mAU/L), NLR (≧4.0 vs. <4.0), number of tumors (≦3 vs. >3), tumor size (≦5 vs. >5 cm), duration of initial HCC to LDLT (<1 vs. ≧1 year), duration of last treatment to LDLT (<3 vs. ≧3 months), Milan criteria (yes vs. no), graft vs. standard liver volume (<35% vs. ≧35%), age of donor (<30 vs. >30 years), histologic number of tumors (<10 vs. ≧10), histologic tumor size (≦5 vs. >5 cm), histologic tumor differentiation (well+moderate vs. poor), histologic vascular invasion (yes vs. no), adjuvant chemotherapy (yes vs. no), CNI (CyA vs. Tac), steroid use (yes vs. no), IFN against HCV (yes vs. no), AFP level at recurrence (≧300 vs. <300 ng/mL), DCP level at recurrence (≧300 vs. <300 mAU/L), NLR at recurrence (≧4.0 vs. <4.0), initial site of recurrence (liver vs. extraliver), duration of LDLT to recurrence (<1 vs. ≧1 year), and surgical resection for recurrent HCC (yes vs. no).

Patient Follow-up

The clinical follow-up of patients transplanted for HCC followed a strict protocol, which did not change during the study period. The patients were seen biweekly for the first month and then screened monthly for 6 months for tumor markers such as AFP and DCP. The patients had ultrasound scans and enhanced computed tomography scans at 6-month intervals. When recurrence was suspected, additional examination such as hepatic angiography were performed. The median follow-up period was 3.9 years.

Treatment of HCC Recurrence

Patients with recurrence that could be surgically cured underwent a resection or ablation of their tumors. All patients considered to be unsuitable for surgical treatment were referred for palliative care by radiotherapy, transarterial chemoembolization, and administration of 5-fluorouracil–based systemic therapy.

Histologic Study

All of the resected specimens were cut into serial 5- to 10-mm-thick slices and fixed in 10% formalin. After macroscopic examination, the slice with the greatest dimensions was trimmed for embedding in paraffin and cut into 4-μm microscopic sections. The sections were stained with hematoxylin-eosin. Tumor differentiation, microvascular invasion, intrahepatic metastasis, and histologic liver cirrhosis were examined by the pathologist according to the Liver Cancer Study Group in Japan (*19*).

Statistical Analysis

We analyzed the categorical clinicopathologic variables using the chi-square test or Fisher's exact test. Continuous variables were expressed as means and SDs and compared with the Student's *t* test. The survival curves after recurrence of the two groups were analyzed by the Kaplan–Meier method and compared with the log-rank test. All analyses were performed with Statview 5.0 software (Abacus Concepts, Berkeley, CA). NLR in a subsequent phase were compared by repeated-measures analysis of variance. $P<0.05$ was considered statistically significant.

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Original article

Sarcopenia as a predictor of prognosis in patients following hepatectomy for hepatocellular carcinoma

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Background: Sarcopenia was identified recently as a poor prognostic factor in patients with cancer. The present study investigated the effect of sarcopenia on short- and long-term outcomes following partial hepatectomy for hepatocellular carcinoma (HCC), and aimed to identify prognostic factors.

Methods: Data were collected retrospectively for all consecutive patients who underwent hepatectomy for HCC with curative intent between January 2004 and December 2009. Patients were assigned to one of two groups according to the presence or absence of sarcopenia, assessed by computed tomographic measurement of muscle mass at the level of the third lumbar vertebra. Clinicopathological, surgical outcome and long-term survival data were analysed.

Results: Sarcopenia was present in 75 (40·3 per cent) of 186 patients, and was significantly correlated with female sex, lower body mass index and liver dysfunction, as indicated by abnormal serum albumin levels and indocyanine green retention test at 15 min values. In patients with, and without sarcopenia, the 5-year overall survival rate was 71 and 83·7 per cent respectively, and the 5-year recurrence-free survival rate was 13 and 33·2 per cent respectively. Multivariable analysis revealed that reduced skeletal muscle mass was predictive of an unfavourable prognosis.

Conclusion: Sarcopenia was predictive of worse overall survival even when adjusted for other known predictors in patients with HCC after partial hepatectomy.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world[1,2]. As a consequence of advances in the diagnosis and management of HCC, major improvements in overall and disease-free survival rates for HCC after partial hepatectomy have been achieved. However, even when curative resection is performed, a considerable number of patients develop intrahepatic or extrahepatic recurrence[3,4]. The prognostic assessment of patients with HCC after hepatic resection and recurrence is an important clinical issue in this population[5–7]. Both tumour- and host-related factors are related to clinical outcome, and general condition and liver function are important in this context. Unfortunately, it is difficult to evaluate the general condition of patients excluding liver function before hepatectomy. Conventional methods, such as the Child–Pugh classification, have been used initially to determine the severity of cirrhosis and to select patients who might tolerate hepatic resection. However, these methods do not reflect the patient's general condition. The American Society of Anesthesiologists (ASA) grade was reported to predict the prognosis of HCC after hepatectomy[8], but this classification is not always objective.

Recently, loss of skeletal muscle mass, termed sarcopenia, was identified as a poor prognostic factor for patients with pancreatic cancer, colorectal liver metastases, melanoma, liver cirrhosis and liver transplantation[9–14]. Sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength, with a risk of adverse outcomes such as physical disability, poor quality of life and death[15,16]. To date, there have been no reports on the relationship between sarcopenia and the prognosis of patients with HCC following hepatic resection.

A retrospective study was performed at the authors' institution to investigate the outcome of patients with sarcopenia who underwent hepatic resection for HCC. The outcome of these patients was compared with that of patients without sarcopenia undergoing hepatic resection during the same period.

Methods

All patients who underwent hepatic resection with curative intent as the initial treatment in the Department of Surgery II, Kyushu University Hospital, between January 2004 and December 2009 were enrolled in the study. Curative resection was defined as complete macroscopic removal of the tumour. All patients had preoperative computed tomography (CT). A transverse CT image at the third lumbar vertebra (L3) in the inferior direction was assessed from each scan. Skeletal muscle was identified and quantified by Hounsfield unit (HU) thresholds of −29 to +150 (water is defined as 0 HU, air as 1000 HU). Multiple muscles were quantified, including the psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal oblique abdominal muscle, and rectus abdominis muscle (*Fig. 1*). CT measurements were calibrated with water and air at fixed intervals. Cross-sectional areas (cm^2) of skeletal muscles in the L3 region were measured by manual outlining on the CT images, and checked by the radiologist. The cross-sectional areas were then normalized for height (cm^2/m^2).

Cut-off values for skeletal muscle associated with overall survival were defined as 43·75 cm^2/m^2 for men and 41·10 cm^2/m^2 for women[10]. Based on this cut-off, patients were assigned to one of two groups, depending on the presence or absence of sarcopenia. The clinicopathological background and rates of overall and recurrence-free survival were compared between the two groups.

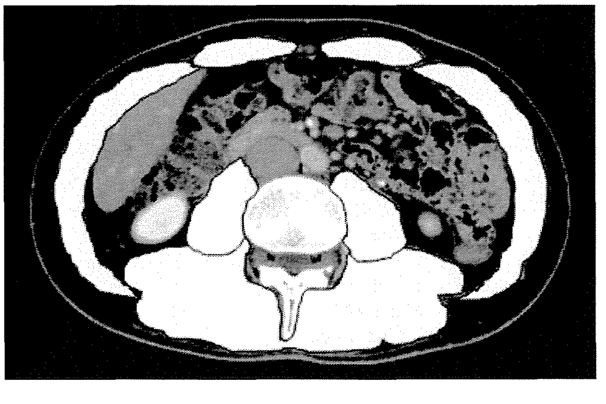

Fig. 1 Computed tomogram showing the area of skeletal muscle mass in the L3 region (highlighted yellow)

The prognostic factors were examined with respect to overall and recurrence-free survival on the basis of the following variables: sarcopenia (absence *versus* presence); skeletal muscle mass; age; sex (male *versus* female); body mass index (BMI); hepatitis B surface antigen (positive *versus* negative), hepatitis C virus antibody (positive *versus* negative); serum albumin level; serum total bilirubin level; serum aspartate aminotransferase level; platelet number; indocyanine green retention test at 15 min (ICGR15); Child–Pugh grade (A *versus* B); Model for End-Stage Liver Disease (MELD) score; histological liver cirrhosis (normal liver + chronic hepatitis *versus* liver fibrosis and liver cirrhosis); tumour size; tumour number (solitary *versus* multiple); tumour node metastasis (TNM) stage according to the Liver Cancer Study Group of Japan[17] (I + II *versus* III + IV); tumour differentiation (well differentiated + moderately differentiated *versus* poorly differentiated); microvascular invasion (MVI) (absence *versus* presence); intrahepatic metastases (absence *versus* presence); serum α-fetoprotein level (AFP); des-γ-carboxyprothrombin (DCP) level; operative procedure (anatomical *versus* non-anatomical resection); duration of surgery; estimated blood loss; and postoperative complications (absence *versus* presence). Patients with diabetes were defined as those using an oral hypoglycaemic agent or insulin. The MELD score was calculated in accordance with a previous report[18]. Postoperative complications within 1 month after partial hepatectomy included liver failure, encephalopathy, gastrointestinal bleeding, intraperitoneal abscess, abdominal haemorrhage, bile leakage, pleural effusion, intractable ascites and wound infection. Complications were classified according to Clavien–Dindo[19]; grade III complications (those requiring surgical intervention) were considered to indicate the presence of a postoperative complication.

Surgical procedures

Details of surgical techniques and patient selection criteria have been reported previously[7]. Selection criteria for hepatic resection were: ascites not detected, or controllable by diuretics; serum total bilirubin level lower than 2·0 mg/ml; and ICGR15 value below 40 per cent. The surgical approach included a J-shaped incision for routine abdominal access, hepatic dissection using an ultrasonic dissector with a coagulator (CUSA EXcel®; Integra, Plainsboro, New Jersey, USA), with systematic ligation of all sizable vessels, and close ultrasonographic guidance along the transection line. Cholecystectomy was performed in all patients if applicable. An intraoperative bile leak test was performed routinely[20]. Small bile leaks on the cut liver surface were repaired by Z-suturing with 6-0 polydioxanone (PDS II; Johnson and Johnson, Tokyo, Japan). Intraoperative vascular control was achieved with the Pringle manoeuvre[21].

Follow-up strategy and recurrence pattern

After discharge, all patients were examined monthly for recurrence by ultrasonography and estimation of tumour markers, such as AFP and DCP, and by CT every 6 months. When recurrence was suspected, additional examinations such as hepatic angiography were performed. Recurrent HCC was treated by repeat hepatectomy, ablation therapy and lipiodolization, as described previously[22].

Histological assessment

All resected specimens were cut into serial 5–10-mm thick slices and fixed in 10 per cent formalin. After macroscopic examination, the slice with the greatest dimensions was trimmed for embedding in paraffin and cut into 4-μm microscopic sections. The sections were stained with haematoxylin and eosin. Tumour differentiation, MVI, intrahepatic metastases and histological liver cirrhosis were assessed by the pathologist in accordance with the rules of the Liver Cancer Study Group of Japan[17].

Table 1 Clinicopathological factors in patients with, and without sarcopenia

	Sarcopenia ($n = 75$)	No sarcopenia ($n = 111$)	P†
Age (years)	67(11)	66(10)	0·553
Sex ratio (M : F)	50 : 25	95 : 16	0·004‡
Skeletal muscle mass (cm^2/m^2)	37·8(3·7)	49·7(6·5)	<0·001
Body mass index (kg/m^2)	20·5(2·4)	24·0(2·8)	<0·001
Diabetes mellitus	22 (29)	35 (31·6)	0·999‡
Albumin (g/dl)	3·8(0·4)	4·0(0·4)	0·002
Total bilirubin (mg/dl)	0·9(0·4)	0·8(0·3)	0·096
Platelet count ($\times 10^4/\mu l$)	15·5(7·5)	16·3(6·2)	0·454
ICGR15 (%)	15·7(8·2)	13·6(6·2)	0·049
Child–Pugh grade			0·190‡
A	68 (91)	107 (96·4)	
B	7 (9)	4 (3·6)	
MELD score	7·7(2·1)	7·9(1·8)	0·591
Hepatitis grade			0·652‡
None	11 (15)	13 (11·7)	
Mild	55 (73)	80 (72·1)	
Severe	9 (12)	18 (16·2)	
Liver cirrhosis			0·290‡
Normal liver + chronic hepatitis	32 (43)	55 (49·5)	
Liver fibrosis + liver cirrhosis	43 (57)	56 (50·5)	
Tumour size (cm)	4·0(3·2)	3·9(2·8)	0·770
No. of tumours			0·171‡
Solitary	52 (69)	88 (79·3)	
Multiple	23 (31)	23 (20·7)	
TNM stage			0·967‡
I	11 (15)	18 (16·2)	
II	38 (51)	57 (51·4)	
III	20 (27)	29 (26·1)	
IV	6 (8)	7 (6·3)	
Differentiation of HCC			0·690‡
Well	9 (12)	10 (9·0)	
Moderate	50 (67)	77 (69·4)	
Poor	16 (21)	24 (21·6)	
Microvascular invasion	24 (32)	37 (33·3)	0·890‡
Intrahepatic metastases	12 (16)	18 (16·2)	0·978‡
α-Fetoprotein level (ng/ml)	3459(18 300)	12 250(70 470)	0·297
DCP (munits/l)	4318(13 627)	2942(12 499)	0·480
Postoperative complications	24 (32)	56 (50·5)	0·613‡

Values are mean(s.d.) unless indicated otherwise: *values in parentheses are percentages. ICGR15, indocyanine green dye retention test at 15 min; MELD, Model for End-Stage Liver Disease; TNM, tumour node metastasis (stage defined by the Liver Cancer Study Group of Japan); HCC, hepatocellular carcinoma; DCP, des-γ-carboxyprothrombin. †Mann–Whitney U test, except ‡Fisher's exact test or χ^2 test.