Title page

Ketone bodies as a predictor of prognosis of hepatocellular carcinoma after transcatheter arterial chemoembolization

Running title: Ketone bodies and muscle status with HCC

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Abbreviations: HCC, hepatocellular carcinoma; 3-OHB, 3-hydroxybutyrate; AcAc, acetoacetate; TACE, transcatheter arterial chemoembolization; CT, computed tomography; MRI, magnetic resonance imaging; TKBR, total ketone body ratio; PMI, psoas muscle mass index; IMAC, intramuscular adipose tissue content; c-TACE, conventional TACE; DEB-TACE, drug-eluting bead TACE; BCLC stage, Barcelona Clinic liver cancer stage

Abstract

OBJECTIVE: Arterial ketone bodies, which reflect liver function, have been investigated. However, the relationship between venous ketone bodies and hepatocellular carcinoma (HCC) is unclear. We investigated whether prognosis of patients with HCC after transcatheter arterial chemoembolization (TACE) was associated with venous blood ketone bodies.

RESEARCH METHODS & PROCEDURES: Sixty-eight patients with HCC who underwent TACE were recruited for this study. The venous blood ketone body levels were measured 1 day before (pre-treatment) and 7 days after TACE (post-treatment). Skeletal muscle quality was evaluated using the intramuscular adipose tissue content (IMAC).

RESULTS: Of the 68 patients, 43 (63.2%) were male with median age of 73.0 years, and the IMAC was -0.274 (range -0.82 to 0.24). The median ketone body levels pre- and post-treatment were 63.0 μmol/L (13-310) and 48.0 μmol/L(8-896), respectively. The cumulative survival rate of patients with total ketone body ratio ([TKBR]: post-treatment/pre-treatment total ketone bodies)<1 was 86.6%. The rate with TKBR≥1 was 59.0% at 300 days (P<0.05). Cox regression analysis identified the TKBR (1≥, hazard ratio: 2.954, 95% confidence interval [CI]: 1.040-8.390, P=0.030) that independently and significantly predicted the patients' prognoses. Logistic regression analysis revealed the IMAC (>-0.2745, odds ratio: 3.958, 95%CI: 1.137-13.779,

P=0.031) that predicted TKBR. TKBR and IMAC were positively correlated (rS=0.358, *P*=0.003).

CONCLUSIONS: The changes in the venous ketone body were associated with the muscle status and predicted the prognosis of patients with HCC who underwent TACE. The venous ketone bodies could be a new predictor of the prognosis of HCC patients after TACE.

Key words: intramuscular adipose tissue content, skeletal muscle quality, liver disease, cirrhosis, cancer

Highlights:

- Venous ketone bodies in HCC patients are useful to predict skeletal muscle quality.
- Increase of venous ketone bodies is negatively correlated with survival.
- Venous ketone bodies could be a predictor of HCC patients' prognosis after TACE.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths globally [1, 2]. Patients with HCC are at an increased risk of being malnourished, and most HCCs develop due to chronic liver diseases and cirrhosis. Malnutrition is a common finding in patients with cancer and cirrhosis [3, 4]. Moreover, sarcopenia is the major component of malnutrition, and it is a frequent complication in patients with chronic liver disease or cirrhosis [4-6]. Nutritional assessment of patients with chronic liver disease or cirrhosis is essential, because malnutrition is an independent predictor of mortality and complications [7-11]. However, there is no simple marker to assess nutrition or sarcopenia in HCC patients with chronic liver disease or cirrhosis.

Ketone bodies are composed of three molecules: 3-hydroxybutyrate (3-OHB), acetoacetate (AcAc), and acetone, which are produced from fatty acids in the liver. Ketone bodies play an important role in survival during starvation and provide a source of energy to the tissues in the brain, heart, muscle, and kidney in patients with glucose insufficiency [12].

Previous studies have shown the relationship between hepatic reserve function and arterial ketone bodies during liver surgery or transcatheter arterial chemoembolization (TACE) [13-17]. However, no studies have focused on venous ketone bodies, nutrition

status, and skeletal muscle status in patients with chronic liver disease, cirrhosis, or HCC. In this report, we utilized venous ketone bodies to assess the nutrition and skeletal muscle status, as well as predict the prognosis of patients with HCC who underwent TACE.

METHODS

Patients

Between June 2014 and May 2015, 133 consecutive HCC patients who underwent TACE at our institution were enrolled in this retrospective study. HCC was diagnosed based on the positive results of typical vascular patterns, revealed by either contrast-enhanced computed tomography (CT), contrast-enhanced magnetic resonance imaging (MRI), or angiography. Otherwise, the pathological diagnosis was made via a fine-needle biopsy of space-occupying lesions that were detected in the liver.

The exclusion criteria for this study were (1) a shorter follow-up period (<2 months) after TACE treatment, (2) absence of properly examined samples or insufficient archival material, and (3) no HCC definite diagnosis. After the exclusion criteria were applied, data on 68 patients who underwent TACE were analysed retrospectively.

Measurement of blood samples and ketone bodies

For all patients in our cohort, a blood sample was collected 1 day before (pre-treatment) and 7 days after (post-treatment) TACE treatment. Medical histories, along with the results of routine tests for blood cell counts, liver biochemistry, and tumour markers at the time of TACE and thereafter were retrieved from the patients' medical records. Complete blood cell counts were obtained and biochemical tests were performed using automated procedures in the clinical pathology laboratories of our hospital. All blood samples were collected with the patients in a fasting state. The total ketone body level, 3-OHB, and AcAc were measured via an enzyme cycling method using a commercial kit (TKB-L Shiyaku Kainos, 3HB-L Shiyaku Kainos; Kainos, Tokyo, Japan). We also calculated the total ketone body ratio (TKBR) by dividing the total ketone bodies at post-treatment (day 7) by the number of total ketone bodies at pre-treatment (day 0).

Image analysis

The quality of the skeletal muscle was evaluated using the psoas muscle mass index (PMI) and intramuscular adipose tissue content (IMAC) using CT imaging. The cross-sectional areas of the right and left psoas muscles were measured with manual tracing on CT imaging at the L3 level to determine the PMI. The PMI was calculated by normalizing the cross-sectional areas for height (cm^2/m^2) [18]. The IMAC was determined by measuring the CT value of the multifidus muscles (Hounsfield units) and

the CT value of subcutaneous fat (Hounsfield units) at the umbilical level. The IMAC was calculated by dividing the region of interest of the multifidus muscle (Hounsfield units) with the region of interest of the subcutaneous fat (Hounsfield units) [19, 20].

TACE treatment

Among the 68 patients, 52 underwent conventional TACE (c-TACE) and 16 underwent drug-eluting bead (DEB)-TACE. The standard TACE procedure was performed via a right femoral artery puncture. Selective arteriography of the celiac and superior mesenteric arteries was performed to investigate the arterial anatomy of the liver, vascular supply of the tumour nodes, and patency of the portal vein. TACE was performed as selectively as possible. A mixture of metal matrix composite (Mitomycin-C; Kyowa, Tokyo, Japan) or epirubicin (Nippon Kayaku, Tokyo, Japan) that was manually emulsified with iodized oil (Lipiodol; Fuji Pharma Co., Tokyo, Japan) was used for the c-TACE procedures. The dosages of the chemotherapeutic drug used were determined according to the patient's body surface area and tumour size. Subsequently, embolization was performed with absorbable gelatin sponge particles (Gelpart; Nippon Kayaku, Tokyo, Japan) to reduce residual blood flow. DEB (DC Bead; Eisai Co, Tokyo, Japan) with a diameter of 100-300, 300-500, and 500-700 μm was used for the DEB-TACE procedures. The dosage of epirubicin was 50 mg, which was

equivalent to one vial of drug-eluting beads. Interventional radiologists clinically decided whether c-TACE or DEB-TACE should be performed.

Follow-up and diagnosis of HCC

All patients were followed up at an interval of 1-3 months, the blood count and liver biochemistry were measured, and the alpha-fetoprotein and des-gamma-carboxy prothrombin levels were quantitatively detected. Diagnostic imaging either by ultrasound, CT, or MRI was performed at least once every3 months.

Ethical considerations

Informed consent to use medical records and specimens was obtained from each patient. These processes and the study protocol were approved by the Ethical Committee of our institution (confirmation number: 16031421), and conformed to the 1975 Declaration of Helsinki and the Japanese Ethical Guidelines for Clinical Research (Ministry of Health, Labour, and Welfare of Japan, Ethical Guidelines for Clinical Research, 2008).

Statistical analysis

Continuous variables (albumin, prothrombin time, total bilirubin, alpha-fetoprotein, Fib-4, PMI, IMAC, and tumour size) were dichotomized with respect to the median value or clinically meaningful values in a multivariate analysis. A statistical analysis

was performed using Wilcoxon's signed rank test and Mann-Whitney's U-test. To estimate the survival rate after TACE, we used the Kaplan-Meier method and the log-rank test. To select the optimal cutoff values of TKBR that indicate a poor prognosis after TACE, the area under the time-dependent receiver operating characteristic curves [21] was assessed. A Cox proportional hazards regression analysis was performed to evaluate the risk factors for survival after TACE. A multiple regression analysis was performed to determine the factors that were associated with TKBR. Age, sex, and variables with *P*-values of <0.20 were selected and entered into the multiple regression model. A *P*-value of 0.05 was considered statistically significant. The data analysis was performed with SPSS ver. 22.0 (SPSS, Chicago, IL, USA).

RESULTS

Patient characteristics

The baseline characteristics of the 68 patients who were included in this study are summarized in Table 1. The median age was 73.0 years; 43 patients (63.2%) were male; branched chain amino acids were administered to 30 (44.1%); and 3 (4.4%), 24 (35.3%), 26 (38.2%), 14 (20.6%), and 1 (1.5%) patients were diagnosed with Barcelona Clinic liver cancer (BCLC) stage 0, A, B, C, and D cancers, respectively. The median PMI was 5.70 (range: 2.39-8.72 cm^2/m^2), the IMAC was -0.274 (range: -0.82 to 0.24); the tumour size was 2.2 (range: 1.0-15.0 cm), 15 (22.1%) patients were affected in a single nodule,

and 21 (30.9%) patients were diagnosed with diabetes mellitus. The median follow-up period was 272.5 (range: 67-595 days).

Total ketone bodies at pre-treatment

The median total ketone body level, 3-OHB, and AcAc at pre-treatment were 63.0 (range: 13-310 μmol/L), 47.5 (range: 7-222 μmol/L), and 18.0 (range: 3-100 μmol/L), respectively (Figure 1). We divided the 68 patients into two groups stratified by the median value of the total ketone body level at pre-treatment. In the group of patients whose total ketone body level was ≥ 63.0 μmol/L, the following two factors were identified as significant in the univariate analysis: IMAC ratio and a history of diabetes mellitus (Table 2).

Chronological changes in the total ketone body levels

Figure 2 shows the changes in the total ketone body levels. The median value of the total ketone body level at pre-treatment was 63.0 (range: 13-310 μmol/L) and that of the total ketone body level at post-treatment was 48.0 (range 8-896 μmol/L). There was no significant change in the total ketone body level between pre- and post-treatment (Figure 2A). However, in 40 patients (58.8%), the total ketone body level after TACE decreased, and there was an increase in these levels in the other 28 patients (Figure 2B). The median value of the pre-treatment ketone body ratio (AcAc/3-OHB) was 0.41

248 (range: 0.1-1.3) and that of post-treatment was 0.45 (range: 0.2-2.3). There was no
249 significant change between the pre- and post-treatment ketone body ratios (data not
250 shown).
251

252 ## *Cumulative survival rate after TACE*

253 During the follow-up period, 16 of the 68 patients (23.5%) died. The cumulative
254 survival rate was 97.1% at 100 days, 86.2% at 200 days, and 76.6% at 300 days. To
255 evaluate the relationship between the total ketone body level and survival after TACE,
256 we characterized these 68 patients according to their total ketone body level at
257 pre-treatment and TKBR (post-treatment/pre-treatment total ketone bodies). Figure 3A
258 shows the survival rate after TACE, stratified by the median value of the total ketone
259 bodies at pre-treatment. There were no significant differences in the survival rate
260 between total ketone bodies ≥63.0 μmol/L group and <63.0 μmol/L group at
261 pre-treatment (P=0.61 in the log-rank test). Figure 3B shows the survival rate after
262 TACE, stratified by the TKBR. The 200, 300, and 400-day survival rates were 86.6%,
263 86.6%, and 81.5%, respectively, in the 40 patients with TKBR<1 (reduced group); and
264 86.0%, 59.0%, and 50.6%, respectively, in the 28 patients with TKBR≥1 (raised group).
265 The survival rates were significantly higher in the reduced group than in the raised
266 group (P<0.05 in the log-rank test).

Risk factors for survival after TACE

The univariate analysis demonstrated the factors that influence the risk for survival after TACE. A Cox regression analysis was performed for 18 variables: age, sex, branched chain amino acid levels, BCLC stage, albumin, prothrombin time, total bilirubin, Child-Pugh grade, alpha fetoprotein, Fib-4 index, PMI, IMAC, tumour size, tumour number, diagnosis of diabetes mellitus, type of TACE, total ketone body level, and TKBR. The following three factors were identified as risk factors for survival after TACE using the univariate analysis (P<0.20): Fib-4 index, tumour size, and TKBR (Table 3).

A multivariate analysis was performed on the three factors (Fib-4 index, tumour size, and TKBR) identified via univariate analysis (P<0.20). TKBR (≥1, hazard ratio: 2.954, 95% confidence interval: 1.040-8.390, P=0.030) was identified as an independent and significant risk factor of patient prognosis after TACE (Table 3).

Predictors for TKBR

We divided the patients into two groups, stratified by TKBR. The following nine significant (P<0.20) factors were identified in the TKBR raised group in the univariate analysis: age, sex, BCLC stage, prothrombin time, total bilirubin, PMI, IMAC, tumour

size, and diagnosis of diabetes mellitus. Multivariable logistic-regression models were prepared to estimate the predictors for the TKBR. A multivariate logistic regression analysis revealed that the IMAC (>-0.2745, odds ratio: 3.958, 95% confidence interval: 1.137-13.779, *P*=0.031) and tumour size (>2.2 cm, odds ratio: 4.115, 95% confidence interval: 1.072-15.796, *P*=0.039) were predictors for the TKBR (Table 4).

Correlation between the TKBR and IMAC

Figure 4 shows a scatter plot of the TKBR and the IMAC. The values that were obtained for the TKBR and IMAC groups in the Spearman's rank order correlation test (rS=0.358) showed that there was a positive correlation between the groups (*P*=0.003).

DISCUSSION

Ketone bodies are small lipid-derived molecules that are a source of energy for the peripheral tissues during fasting or prolonged exercise [22]. During fasting, the muscle and liver stores of glycogen are depleted first. Then, fatty acids are transported to the liver for conversion to ketone bodies. Patients with advanced liver disease particularly have increased fat oxidation [11]. The production of ketone bodies plays an important role in metabolites. Serum ketone bodies are defined by a variety of factors, such as energy metabolism, circumstances (starving, prolonged exercise, low-carbohydrate diets, and diabetes mellitus), liver conditions, and the extra-hepatic tissues (heart, kidney,

brain, and skeletal muscle). Previous studies have focused on arterial ketone bodies and liver function [14, 16]. Conversely, the extra-hepatic tissues are assumed to influence venous ketone bodies.

The first main finding of our study was that venous ketone bodies had different dynamics from those of arterial ketone bodies. Previous studies showed a relationship between the arterial ketone body ratio (AcAc/3-OHB) and hepatic reserve function [15, 16]. However, there was no relationship between venous ketone bodies and hepatic reserve function in our study (Tables 2 and 4). Furthermore, there was no significant change in the venous ketone body ratio (AcAc/3-OHB) after TACE. Recent studies reported that ketone bodies regulate metabolism and 3-OHB signals via extracellular receptors, and endogenously inhibit histone deacetylases [23]. Suppression of oxidative stress due to 3-OHB may benefit organs after a patient undergoes TACE. In our study, in more than half of the patients, the total ketone body level decreased after TACE. Thus, the elevation of ketone bodies 7 days after TACE does not solely reflect ischemic changes.

The second main finding of our study was that the TKBR can predict the patient's prognosis after undergoing TACE. We were able to stratify the patients into different risk groups using the TKBR (Figure 3). The multivariate analysis revealed that the

TKBR was the most significant factor for survival after TACE (Table 3). There was no correlation between the TKBR and hepatic functional reserve. The reason why TKBR predicts prognosis was unclear. However, our study revealed that elevation of the total ketone body level was related to an extra-hepatic factor (skeletal muscle).

The third main finding of our study was that the quality of the skeletal muscle (IMAC) affected the ketone body level after TACE. The multivariate analysis revealed that the quality of the skeletal muscle (IMAC) was the significant factor that predicted the TKBR (Table 4). We observed that the TKBR significantly correlated with the IMAC (Figure 4). A high TKBR allowed us to identify patients with low muscle quality. Skeletal muscle depletion, which indicates a low quantity and quality of skeletal muscle, is referred to as sarcopenia and predicts mortality in patients with advanced liver disease [24-26]. Based on these considerations, we suggest that the TKBR does not reflect the hepatic reserve function, but rather the nutritional status of patients with HCC who underwent TACE. Venous ketone bodies may be associated with nutritional status and sarcopenia in HCC patients.

The present study was limited by its retrospective nature. A future prospective analysis is needed to validate the efficacy of the total ketone body ratio to predict patients' prognoses after undergoing TACE. Another limitation is that there were no definite

criteria to estimate the quantity and quality of the skeletal muscle. In addition, the ketone bodies were influenced by several factors (food intake, administration of drugs, and exercise). Other elements, which we did not evaluate, might have affected the ketone body level. Impaired performance status, advanced stages of disease, and poor hepatic reserve function were associated with shorter survival of patients with HCC [27]. In our study, the Child-Pugh grade or BCLC stage was not a significant factor for survival in patients who underwent TACE. One plausible explanation is that our study consisted of patients with similar backgrounds regarding liver function and disease progression. Furthermore, the small number of participants in this study is also a limitation.

Regardless of these limitations, this is the first report to confirm the relationship between venous ketone bodies and treatment of HCC. In addition, the TKBR may predict the patient's prognosis and be related to the quality of skeletal muscle. Several studies reported that the quantity and quality of the skeletal muscle are important for achieving good clinical outcomes in patients with advanced liver diseases [5, 24-26, 28]. Increasing the skeletal muscle mass and function may be a possible therapeutic target to improve the prognosis of patients with advanced HCC. Therefore, the prediction of patients with poor prognosis after treatment is of increasing clinical relevance.

CONCLUSION

In conclusion, this study revealed that there was an association between venous ketone bodies and survival of HCC patients who underwent TACE. Furthermore, the venous ketone bodies in HCC patients who underwent TACE were useful to predict skeletal muscle quality. The results suggest that venous ketone bodies could be a new predictor of prognosis of HCC patients after TACE.

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Table 1. Characteristics of the patients enrolled in the present study

Factor	N (range, %)
Age, y	73.0 (53-86)
Sex, male	43 (63.2%)
Period, days	272.5 (67-595)
BCAA, +	30 (44.1%)
BCLC stage 0/A/B/C/D	3/24/26/14/1
Albumin, g/dL	3.30 (2.0-4.3)
PT-INR	1.110 (0.95-2.06)
Total bilirubin, mg/dL	0.80 (0.3-2.2)
Child-Pugh grade A/B/C	44/23/1
AFP, ng/mL	23.45 (1.6-20182.0)
Fib-4 index	5.98 (1.2-14.0)
PMI, cm^2/m^2	5.7005 (2.392-8.729)
IMAC ratio	-0.2745 (-0.827-0.239)
Tumour size, cm	2.20 (1.0-15.0)
Tumour number, single	15 (22.1%)
Aetiology B/C/NBNC/alcohol	10/34/13/11
DM	21 (30.9%)
TACE (conventional/DEB)	52/16

Data are given as the medians with ranges. Data were collected at pre-treatment. Abbreviations: BCAA, branched chain amino acids; BCLC stage, Barcelona Clinic liver cancer stage; PT, prothrombin time; AFP, α-fetoprotein; PMI, psoas muscle mass index; IMAC, intramuscular adipose tissue content; HBV, hepatitis B virus; HCV, hepatitis C virus; DM, diabetes mellitus; TACE, transcatheter arterial chemoembolization; DEB, drug eluting beads.

Table 2. Characteristics of the two groups, stratified by the median value of total ketone body level at pre-treatment

Factor	Total ketone bodies <63 μmol/L (n=32)	Total ketone bodies ≥63 μmol/L (n=36)	*P*
Age, years	73.5 (60-86)	73.0 (53-85)	0.60
Sex, male/female	19/13	24/12	0.53
BCAA, -/+	18/14	20/16	0.95
BCLC stage 0/A/B/C/D	2/12/12/5/1	1/12/14/9/0	0.45
Albumin, g/dL	3.30 (2.0-4.2)	3.30 (2.6-4.3)	0.63
PT-INR	1.105 (0.95-2.06)	1.110 (0.97-1.71)	0.94
Total bilirubin, mg/dL	0.80 (0.4-2.0)	0.90 (0.3-2.2)	0.27
Child-Pugh grade A/B/C	17/14/1	27/9/0	0.12
AFP, ng/mL	13.00 (1.9-16326.0)	26.75 (1.6-20182.0)	0.27
Fib-4 index	6.69 (1.4-14.0)	5.80 (1.2-13.9)	0.34
PMI, cm^2/m^2	5.2110 (2.392-7.546)	5.8340 (3.285-8.729)	0.07
IMAC ratio	-0.2335 (-0.53-0.239)	-0.3425 (-0.827-0.056)	< 0.01
Tumour size, cm	2.10 (1.0-5.0)	2.20 (1.0-15.0)	0.27
Tumour number, single/multiple	9/23	6/30	0.25
Aetiology HBV/HCV/non B non C/alcohol	2/20/6/4	8/14/7/7	0.84
DM, -/+	26/6	18/18	< 0.01
TACE conventional/DEB	24/8	28/8	0.78

Data are given as the medians with ranges. Data were collected at pre-treatment. Abbreviations: BCAA, branched chain amino acids; BCLC stage, Barcelona Clinic liver cancer stage; PT, prothrombin time; AFP, α-fetoprotein; PMI, psoas muscle mass index; IMAC, intramuscular adipose tissue

content; DM, diabetes mellitus; TACE, transcatheter arterial chemoembolization; DEB, drug eluting beads. A chi-squared and Mann-Whitney's U tests were performed for comparisons.

Table 3. Risk factors associated with survival after TACE

		Univariate analysis			Multivariate analysis
Risk factor		*P*	Hazard ratio	(95% confidence interval)	*P*
Age, years	>73	0.21			
Sex	Male	0.74			
BCAA	+	0.22			
BCLC stage	B/C/D	0.35			
Albumin, g/dL	>3.3	0.81			
PT-INR	<1.110	0.38			
Total bilirubin, mg/dL	>0.8	0.65			
Child-Pugh grade	B/C	0.92			
AFP, ng/mL	>23.45	0.87			
Fib-4 index	>6.0	0.19	0.522	(0.183-1.485)	0.223
PMI, cm^2/m^2	<5.70	0.38			
IMAC ratio	>-0.2745	0.48			
Tumour size. Cm	>2.20	0.11	1.272	(0.459-3.520)	0.642
Tumour number	Multiple	0.63			
DM	+	0.77			
TACE	DEB	0.33			
Total ketone bodies, μmol/L	≥63.0	0.61			
TKBR	≥1	<0.01	2.954	(1.040-8.390)	0.03

Table 3. Risk factors associated with survival after TACE Hazard ratios for the development of hepatocellular carcinoma were calculated using Cox proportional hazards analysis. Abbreviations: BCAA, branched chain amino acids; BCLC stage, Barcelona Clinic liver cancer stage; PT, prothrombin time; AFP, α-fetoprotein; PMI, psoas muscle mass index; IMAC, intramuscular adipose tissue content; DM, diabetes mellitus; TACE, transcatheter arterial chemoembolization; DEB, drug eluting beads; TKBR, total ketone body ratio.

Table 4. Predictors of the total ketone body ratio (total ketone bodies on day 7/total ketone bodies on day 0)

		Multivariate model		
Independent variables		Odds ratio	(95% confidence interval)	*P*
Age, years	≤73	1	(reference)	
	>73	1.319	(0.416-4.182)	0.638
Gender	Male	1	(reference)	
	Female	1.551	(0.376-6.398)	0.544
BCLC stage	0/A	1	(reference)	
	B/C/D	0.641	(0.162-2.543)	0.527
PT-INR	≤1.110	1	(reference)	
	>1.110	0.682	(0.210-2.221)	0.526
Total bilirubin, mg/dL	≤0.8	1	(reference)	
	>0.8	0.325	(0.096-1.101)	0.071
PMI, cm^2/m^2	≥5.70	1	(reference)	
	<5.70	2.426	(0.651-9.035)	0.187
IMAC	≤-0.2745	1	(reference)	
	>-0.2745	3.958	(1.137-13.779)	0.031
Tumour size, cm	≤2.2	1	(reference)	
	>2.2	4.115	(1.072-15.796)	0.039
DM	-	1	(reference)	
	+	0.823	(0.226-3.001)	0.768

Multivariable logistic-regression models were used to estimate the predictors for the total ketone body ratio. Variables were included in the model based on the univariate analysis ($P<0.20$). Abbreviations: PMI, psoas muscle mass index; IMAC, intramuscular adipose content; DM, diabetes mellitus.

Figure legends

Figure 1: Scatter plots of venous total ketone bodies, 3-hydroxybutyrate, and acetoacetate

The median values are indicated by the horizontal bars in the scatter plot. In the box plot, the bottom and top of the box are the 25th and 75th percentiles (the lower and upper quartiles), respectively.

Figure 2: Chronological changes in the total ketone body levels

Chronological changes in the total ketone body level at pre-treatment (day 0) and post-treatment (day 7) of the 68 HCC patients who underwent TACE. (a): The total ketone bodies were not significantly changed after treatment. The dots represent the median serum total ketone body values at each time point, and the error bar represents the interquartile range. (b): The changes in the total ketone body level in individual patients. The solid line indicates the group of patients in whom the level increased (n=28). The dashed line indicates those in whom the level decreased (n=40). Wilcoxon's signed-rank test was performed for comparisons.

HCC, hepatocellular carcinoma; TACE: transcatheter arterial chemoembolization

Figure 3: Overall survival of HCC patients after undergoing TACE

Overall survival according to (a) the total ketone body values at pre-treatment and (b)

the TKBR. The survival rates were analysed using the Kaplan-Meier method. The black solid lines indicate the stratified (a) total ketone body values at pre-treatment that were ≥ 63 μmol/L and<63 μmol/L, and (b) the TKBR≥1 and <1, respectively. (b) The incidence rate differed significantly between the two groups (P<0.05, in the log-rank test).

HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization; TKBR, total ketone body ratio

Figure 4: Correlation between the TKBR and IMAC

Scatter plot of the TKBR and IMAC. The values that were obtained for the TKBR and IMAC groups using Spearman's rank order correlation test (rS=0.358) showed the presence of positive correlations between the groups (P=0.003).

TKBR, total ketone body ratio; IMAC; intramuscular adipose content

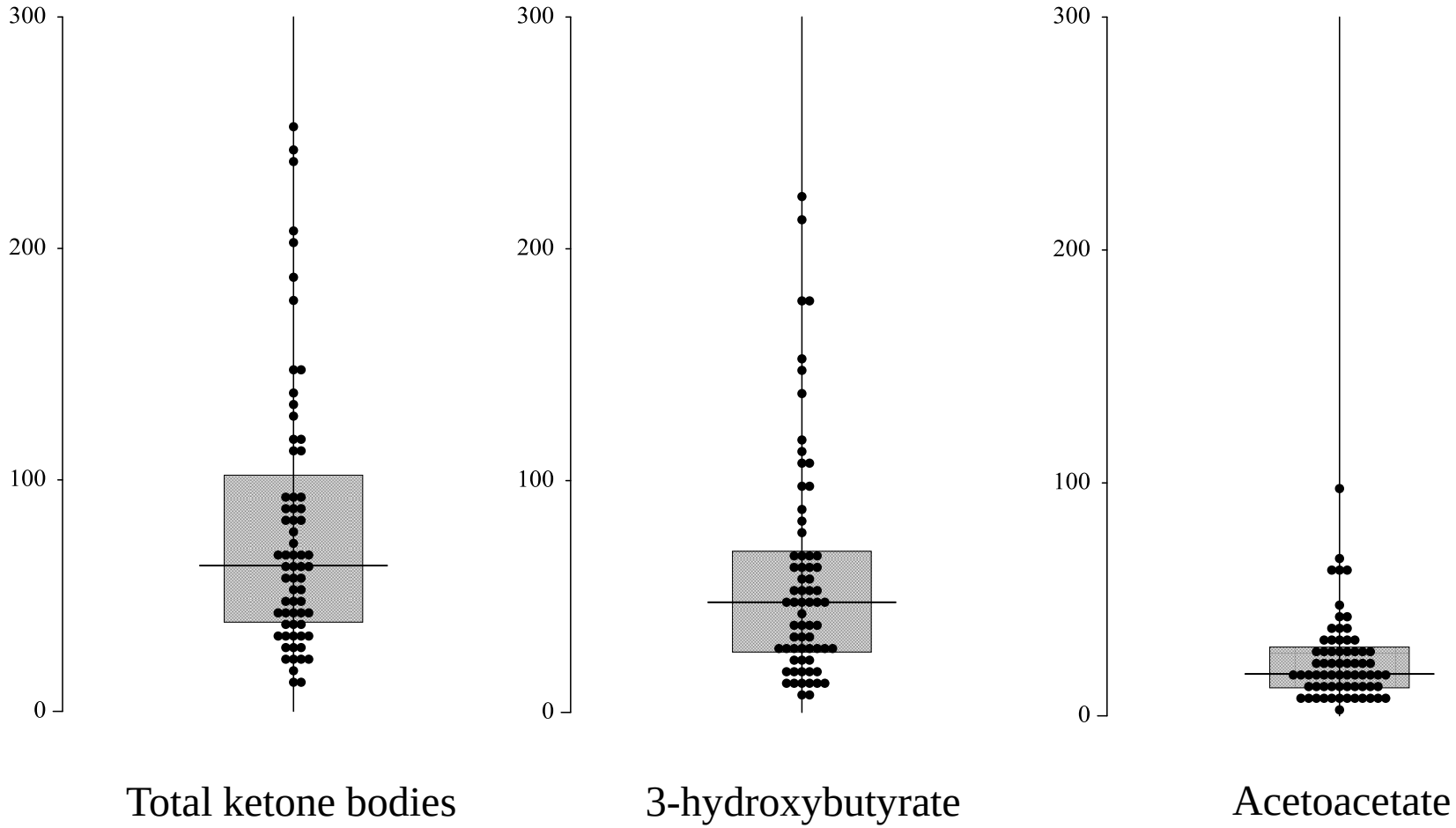


Figure 1. Scatter plot of venous total ketone bodies, 3-hydroxybutyrate and acetoacetate

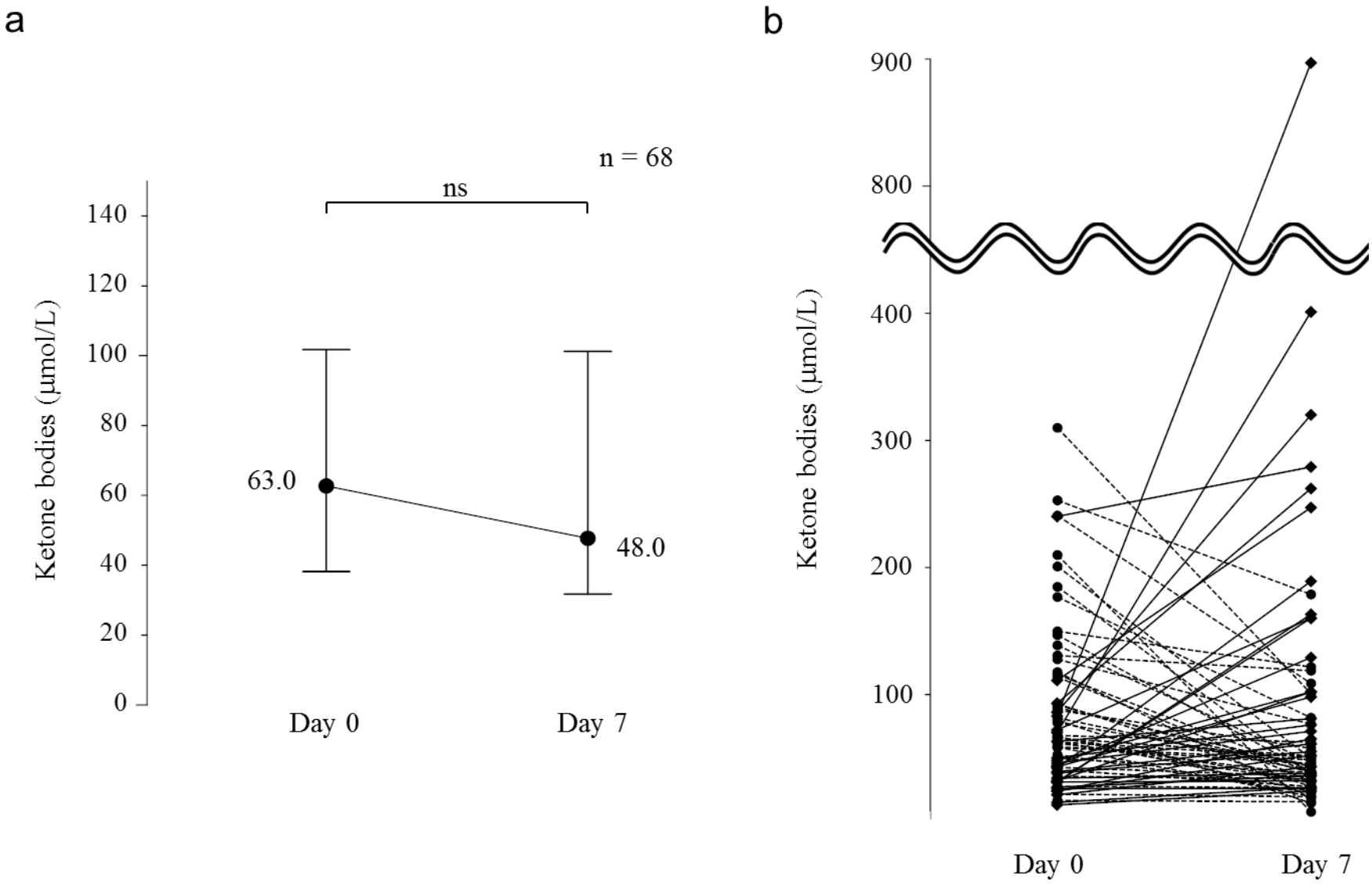
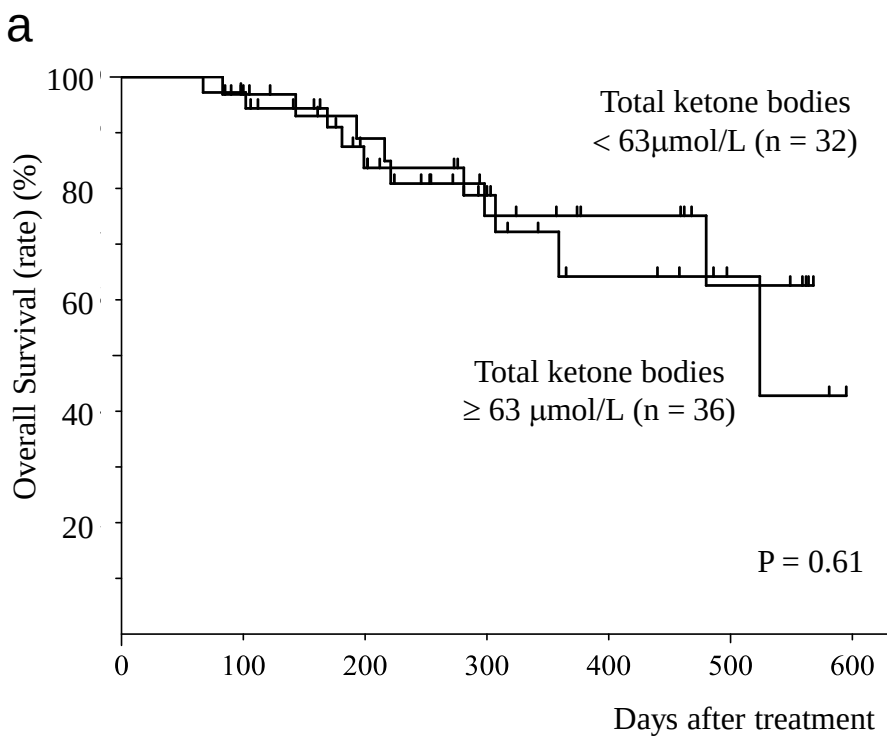
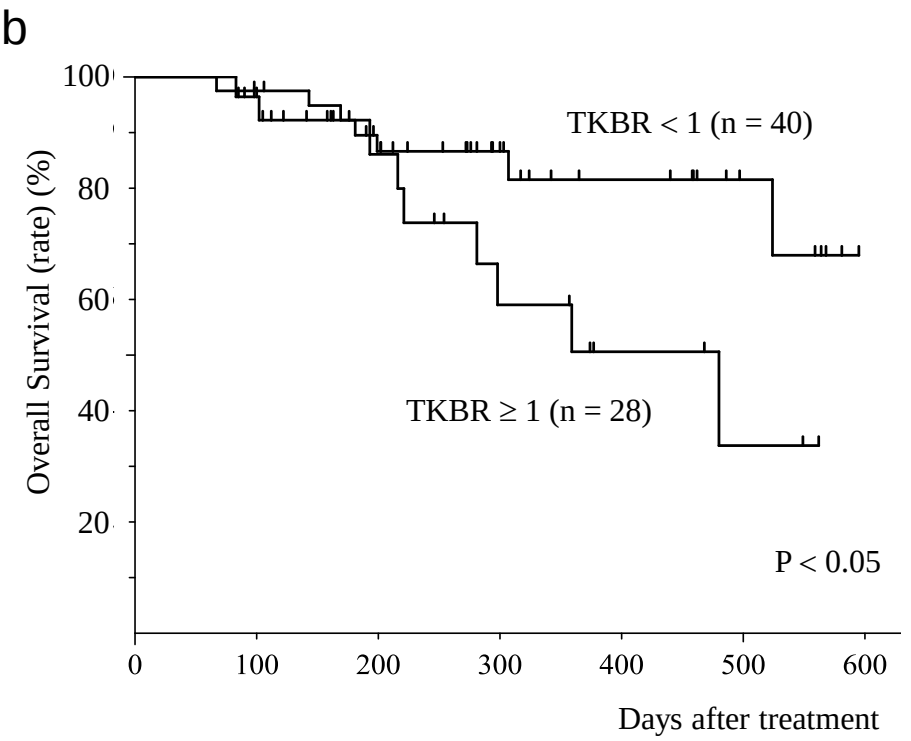


Figure 2. Chronological changes in the total ketone bodies and ketone body levels





Overall Survival (rate) (%)	100-day	200-day	300-day	400-day
Total ketone bodies < 63 μmol/L (n = 32)	96.9%	88.9%	75.1%	75.1%
Total ketone bodies ≥ 63 μmol/L (n = 36)	97.2%	83.6%	78.7%	64.1%

Overall Survival (rate) (%)	100-day	200-day	300-day	400-day
TKBR< 1 (n = 40)	97.5%	86.6%	86.6%	81.5%
TKBR ≥ 1 (n = 28)	96.4%	86.0%	59.0%	50.6%

Figure 3. Overall survival of HCC patients after TACE

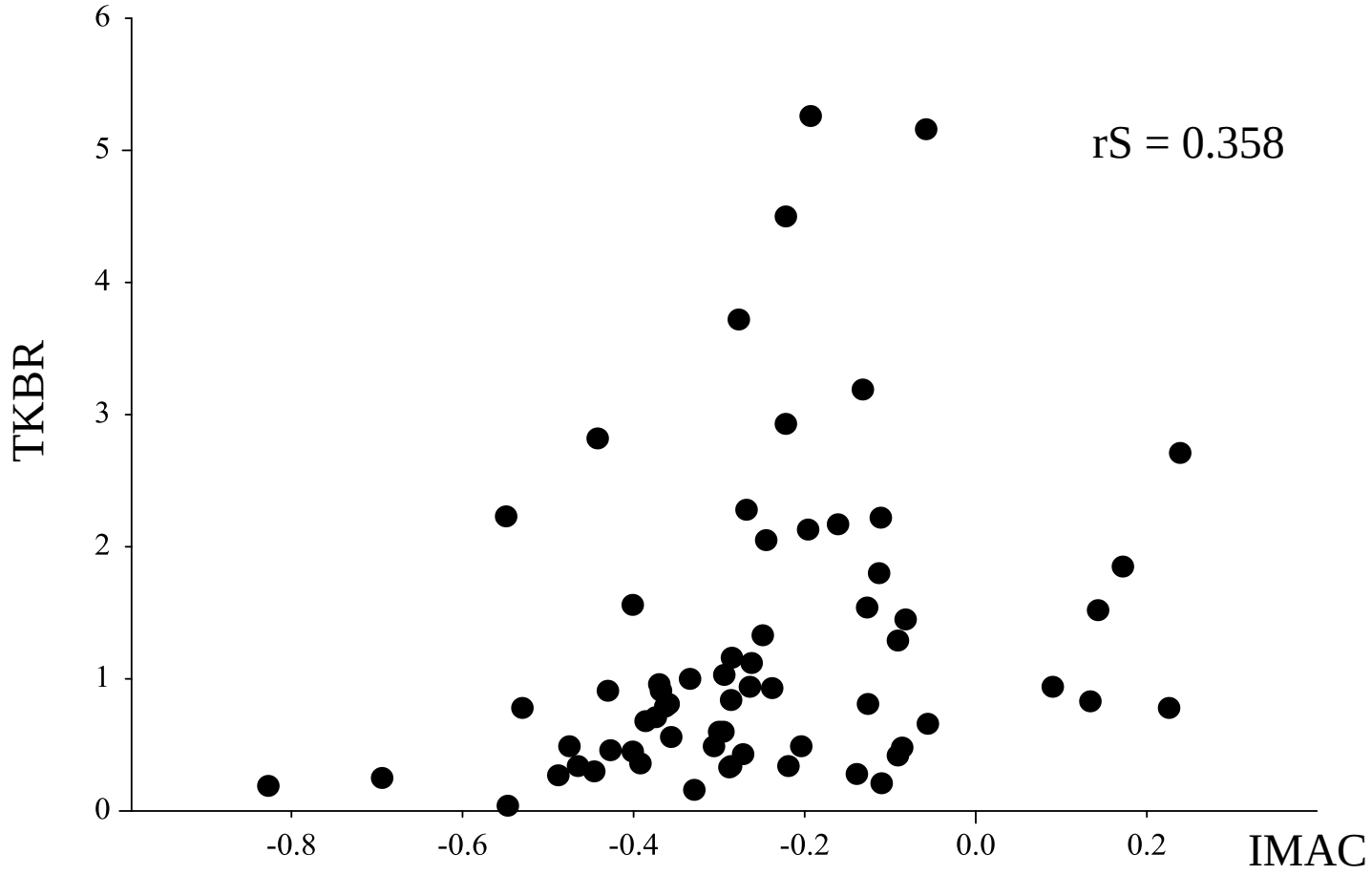


Figure 4. Correlation between the total ketone bodies ratio and intramuscular adipose content