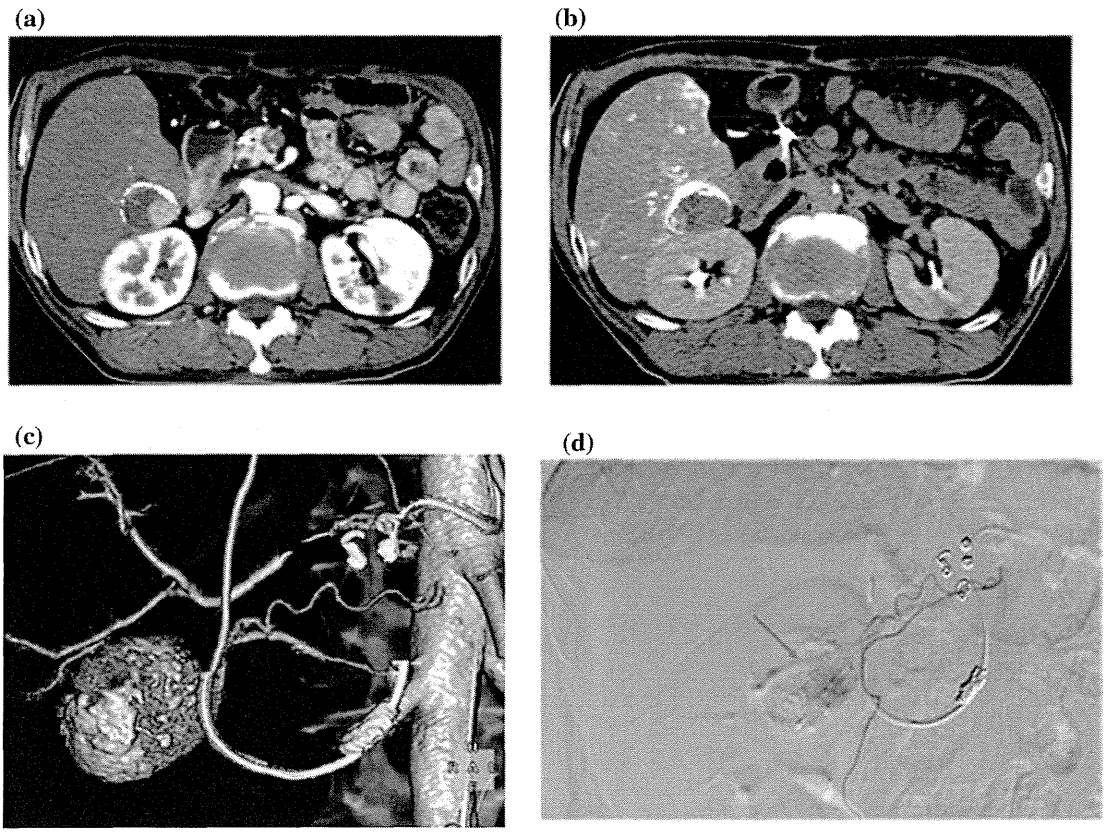

Fig. 3 Identification of extrahepatic hepatocellular carcinoma (HCC) feeding arteries using three-dimensional (3D) vascular images from a combined 64-multidetector-row computed tomography (CT) and angiography system. Whereas HCC recurrence was depicted as enhancement on CT aortography (**a**, *red arrow*), no enhancement was observed in the same area on CT during hepatic arteriography (**b**). This indicated that this HCC was not fed by the hepatic artery, but instead was fed by an extrahepatic source. Analysis of 3D vascular images clearly showed that a very small unnamed artery branching directly from the aorta was feeding this recurrent HCC (**c**, *blue vessel*). Digital subtraction angiography of this artery showed tumor staining consistent with HCC recurrence (**d**)

aortography just after placement of the sheath into the femoral artery contributed more to the accurate and sufficient identification of hepatic and extrahepatic feeding arteries than dynamic CT with intravenous administration of contrast medium before TACE.

There are several weaknesses in this procedure. Extrahepatic feeding arteries with origins outside the CT aortography field, such as the internal thoracic artery and some intercostal arteries, cannot be depicted completely, especially at their origins, so another imaging study may be required to identify the origin. Indeed, we performed repeated CT aortography in one patient, whose HCC was fed by the right internal thoracic artery. CT aortography is necessary for the construction of 3D vascular images, which increases radiation exposure and the use of contrast medium before the start of the TACE procedure. In addition, the present study has several limitations. The numbers of DSA and CT examinations may not strictly reflect the amount of radiation exposure and the amount of contrast medium required during TACE that is actually important for patients. The time required for TACE, which might be associated with the amount of radiation exposure and the amount of contrast medium, tended to be shorter in patients who underwent TACE with angio-MDCT when it was divided by the number of target feeding arteries. However, the time needed for TACE was influenced not only by the rapidness and the accuracy of the identification of the feeding artery, but also by the localization of the target tumor and the degree of difficulty of catheterization, which partly depends on the shape of the target arteries. Furthermore, the skill of the operators for TACE can influence the time needed for TACE, although there was no difference in the time needed for TACE between operators in the present study (data not shown). Therefore, we evaluated the benefit of the angio-MDCT system from the aspect of identification of feeding arteries on the basis of the number of DSA and CT studies. Further studies will be required to confirm the benefit of angio-MDCT for the entire TACE procedure. The treatment effects of TACE were evaluated on the basis of the deposition of iodized oil covering the

entire tumor by unenhanced CT (as an end point for the complete TACE session) and on the basis of the lack of arterial blood supply within the treated HCC by post-TACE dynamic magnetic resonance imaging. However, prospective follow-up for local recurrence after TACE will be necessary to confirm the actual response of TACE. Finally, the number of study patients was small, and larger studies will be required to confirm the benefit of this apparatus.

In conclusion, the novel angio-MDCT system facilitates the rapid and accurate identification and confirmation of HCC feeding arteries that should be catheterized for chemoembolization, and can thus contribute to TACE for treating HCC. Although TACE using C-arm CT [5–8] and single-row angio-CT [10] has improved the identification and confirmation of feeding arteries, angio-MDCT can further improve TACE by reducing radiation exposure and contrast medium use.

Conflict of interest The authors declare that they have no conflict of interest.

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Title page

Impact of the BTR and BCAA granule therapy in patients with HCC: a propensity score analysis

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Footnote page

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List of abbreviations: HCC, hepatocellular carcinoma; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization; BCAA, branched-chain amino acid; AAA, aromatic amino acid; BTR, branched-chain amino acid to tyrosine ratio; AUC, area under the curve; ROC, receiver operating characteristic; CI, confidence interval; CT, computed tomography; MRI, magnetic resonance imaging; AFP, α-fetoprotein; ICD, International Statistical Classification of Diseases and Related Health Problems ; IFN, interferon; HCV, hepatitis C virus; HR, hazard ratio

Abstract

Background and Aim

It has been reported that the branched-chain amino acid (BCAA) to tyrosine ratio (BTR) is a useful indicator of liver function and BCAA therapy is associated with a decreased incidence of hepatocellular carcinoma (HCC). However, there has not been sufficient research on the relationship between BTR and the effects of BCAA therapy after initial treatment of HCC. We investigated the impact of BTR and BCAA therapy on survival in patients with HCC.

Methods

A total of 315 patients with HCC who were treated (n=66) or not treated (n=249) with BCAA were enrolled; of these, 66 were selected from each group using propensity score matching. Survival from liver-related mortality was analyzed.

Results

In patients who did not receive BCAA therapy (n=249), multivariate analysis for factors associated with survival indicated that low BTR (≤4.4) was independently associated with poor prognosis in patients with HCC (hazard ratio [HR], 1.880; 95% confidence interval [CI], 1.125–3.143; p=0.016). In addition, among patients selected by propensity score matching (n=132), multivariate analysis indicated

that BCAA therapy was independently associated with good prognosis in patients with HCC (HR, 0.524; 95% CI, 0.282–0.973; p=0.041). BTR was not significantly associated with survival.

Conclusions

Intervention involving BCAA therapy improved survival in patients with HCC versus untreated controls, regardless of BTR. In addition, low BTR was associated with poor prognosis in patients who did not receive BCAA therapy.

Key Words: branched-chain amino acid to tyrosine ratio, branched-chain amino acid granules, hepatocellular carcinoma, propensity score

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third most common cause of cancer-related death worldwide [1, 2]. With advances in diagnostic imaging along with increased understanding of high-risk patients, HCC can now often be detected at an early stage [3]. In addition to surgical resection as a treatment option for HCC, techniques that can be used alone or in combination include radiofrequency ablation (RFA) and transcatheter arterial chemoembolization (TACE). However, HCC recurrence occurs frequently in the early post-treatment period even in patients who have undergone surgical resection or interventional treatments such as RFA or TACE, because HCC arises in patients with cirrhosis or chronic hepatitis with severe fibrosis. Therefore, despite initial remission of HCC after surgical or other interventional treatments, there are limits to the prolongation of survival. Reasons for poor survival include frequent intrahepatic distant recurrence and, even more importantly, decompensation due to decreases in hepatic functional reserve that accompany progression of chronic liver disease.

The Child-Pugh classification system [4] is the most extensively used method worldwide for assessing hepatic function in patients being treated for HCC. In the Child-Pugh classification system, serum albumin is used to achieve accurate

assessment of protein metabolism status. However, to date, sufficient attention has not been given to the status of amino acid metabolism in chronic liver disease and HCC. Amino acid abnormalities are reportedly common, even in patients with cirrhosis but no hepatic encephalopathy and in patients with chronic hepatitis [5]. The amino acid molar ratio, called Fischer's ratio, is the ratio of branched chain amino acids (BCAAs) [leucine, valine, and isoleucine] to aromatic amino acids (AAAs) [phenylalanine and tyrosine]. This ratio is important for assessing liver metabolism, hepatic functional reserve, and the severity of liver dysfunction [6]. Protein malnutrition is one result of amino acid imbalance. Accordingly, to accurately assess the status of protein metabolism in HCC patients with a background of chronic liver disease, determining the status of amino acid metabolism in addition to the serum albumin level is essential. The BCAA to tyrosine ratio (BTR), which is considered a surrogate for Fischer's ratio, can now be rapidly measured [7].

BCAA granules, which consist of leucine, isoleucine, and valine, have been used in patients with cirrhosis to improve protein malnutrition [8]. A large-scale clinical study has demonstrated the usefulness of BCAA granules in patients with low BTR [9]. Additionally, several clinical studies have also reported that long-term administration of BCAA granules was associated with a decreased incidence of HCC

[10, 11]. However, sufficient research on the relationship between BTR and the effects of BCAA granule therapy and survival in patients with treated HCC has not been carried out.

In the present study, we first investigated the impact of BTR on survival due to liver-related mortality in patients with naïve HCC. In addition, we confirmed the impact of BCAA granules on liver-related mortality in patients with naïve HCC with the use of propensity score matching to reduce biases associated with the selection of study patients [12-15].

Materials and Methods

Patients

Naïve HCC was diagnosed in a total of 1175 patients at the Ogaki Municipal Hospital between January 2000 and December 2013. Of these 1175 patients, 315 met the following eligibility criteria: (i) Child-Pugh class A or B disease; (ii) BTR data at the time of naïve HCC diagnosis was available; (iii) treatment for HCC was performed; (iv) surveillance for HCC recurrence during the follow-up period was performed; (v) duration of follow-up period more than 3 months; (vi) no administration of BCAA granules at the start of follow-up (patients in the BCAA group); (vii) administration of BCAA granules was started during the follow-up period (patients in the BCAA group); (viii) administration of BCAA granules for more than 3 months (patients in the BCAA group); (ix) absence of uncontrollable ascites, edema, or encephalopathy. The start of the follow-up period was defined at the time when naïve HCC was diagnosed. The end of follow-up was defined as the final visit for patients who have not died or patients who died due to non–liver-related disease, or as the date of death for patients who died due to liver-related disease during the follow-up period.

Of these 315 patients, 66 patients received BCAA granules (BCAA group), and the other 249 patients did not receive BCAA granules (non-BCAA group). We first

analyzed survival from liver-related mortality in patients who did not receive BCAA granules (Cohort 1). To reduce the confounding effects of covariates, we used propensity scores to match BCAA patients to unique non-BCAA patients. Nine covariates, age, sex, HCC etiology, albumin level, total bilirubin level, platelet count, α-fetoprotein (AFP) level, HCC stage, and initial HCC treatment modality were taken into account at the start of follow-up. Based on the previously reported cut-off values for administering BCAA granules or the relationship to progression in patients with HCC [11, 16-18], we computed propensity scores using logistic regression with the following independent variables: age (<65 years or ≥65 years), sex (female or male), HCC etiology (hepatitis B; C; or non-B, non-C), albumin level (<3.6 g/dl or ≥3.6 g/dl), total bilirubin level (<1.2 mg/dl or ≥1.2 mg/dl), platelet count (<100 $\times 10^3/\mu l$ or ≥100 $\times 10^3/\mu l$), AFP level (<100 ng/ml or ≥100 ng/ml), HCC stage according to the TNM classification by Liver Cancer Study Group of Japan (I, II, III, or IVA) [19] (Table 1), and initial HCC treatment modality (resection, RFA, TACE+RFA, or TACE). The calculated propensity scores of the BCAA and non-BCAA groups were 0.05114–0.41773 (median, 0.41770) and 0.05114–0.41773 (median, 0.14580), respectively; these scores were then rounded to two decimal places. We conducted one-to-one matching of patients based on propensity scores to the second decimal place.

Propensity score matching resulted in the selection of 132 patients (BCAA group, 66 patients; non-BCAA group, 66 patients) (Cohort 2) (Figure 1). The p-value of the calculated propensity score based on the Hosmer-Lemeshow test was 0.742 [20]. The area under the curve (AUC) of the receiver operating characteristic (ROC)–calculated propensity score was 0.734 (95% confidence interval (CI), 0.670–0.798) [21]. We then analyzed survival from liver-related mortality in these patients matched by propensity scores. The study protocol was in compliance with the Helsinki Declaration and was approved by the institutional review board. Written informed consent for use of their laboratory data was obtained from all patients prior to the study.

Diagnosis and treatment of HCC

HCC was diagnosed based on the results of abdominal ultrasonography and dynamic computed tomography (CT) (hyperattenuation during the arterial phase in part or all of the tumor and hypoattenuation in the portal venous phase), magnetic resonance imaging (MRI), or both, as recommended by the American Association for the Study of Liver Diseases [22]. Arterial and portal phase dynamic CT or MRI was obtained up to 30 and 120 sec after injection of contrast material. For all patients, abdominal angiography combined with CT was performed before treatment for HCC.

We confirmed HCC with CT during hepatic angiography and CT during arterial portography. Regarding HCC treatment, the most appropriate treatment modality for each patient was selected through discussion between surgeons, hepatologists, and radiologists [23-25]. In the present study, there were no patients treated with liver transplantation and there were no treatment-related deaths.

BCAA granule therapy

Patients with HCC were determined as an indication of BCAA treatment when hypoalbuminemia (serum albumin levels of less than 3.6g/dl) was observed during follow-up period after treatment of HCC. For each patient with hypoalbuminemia, the attending physician determined whether treatment with BCAA granules would be performed with patient input after sufficient information regarding BCAA treatment was provided. In the BCAA group, oral BCAA granules (Livact®; Ajinomoto Pharmaceuticals, Tokyo, Japan), containing 952 mg of L-isoleucine, 1904 mg of L-leucine, and 1144 mg of L-valine per sachet, were prescribed to subjects at 4.15g/sachet three times daily after meals [9, 10]. We confirmed that patients in the BCAA group were prescribed BCAA granules regularly by medical record review.

Surveillance for HCC recurrence after initial treatment for HCC

Follow-up observation consisted of regular blood tests and monitoring of tumor markers, including AFP, *lens culinaris* agglutinin-reactive AFP, and des-γ-carboxy-prothrombin every 3 months. Dynamic CT, MRI, or both were performed every 3–4 months after initial treatment for HCC. In particular, for patients in the BCAA group, we confirmed that BCAA granules were being taken properly at each hospital visit. When HCC recurrence or disease progression was detected based on radiologic findings, the most appropriate therapy was initiated in each patient.

Causes of liver-related death

Cause of death were determined by hepatologists in the case of liver-related disease or appropriate specialists in the case of non-liver related disease using International Statistical Classification of Diseases and Related Health Problems (ICD) codes (ICD-9 codes for deaths occurring prior to 1 January 2003, and ICD-10 codes thereafter) [26]. In the present study, we defined death due to HCC, hepatic failure, or esophageal or gastric varices with bleeding as liver-related death. All analyses were performed retrospectively by collecting and analyzing data from patient medical records.

Statistical Analysis

Continuous variables are expressed as medians (range). The Mann-Whitney U test was used for continuous variables, and the chi-square test with Yates' correction or Fisher's exact test was used for categorical variables. Actuarial analysis of cumulative survival due to liver-related mortality was performed using the Kaplan-Meier method, and differences were tested with the generalized Wilcoxon test. Cox proportional hazards models with forward selection (enter and exit probabilities of p=0.1) were used for multivariate analysis of factors associated with survival from liver-related mortality.

Discrimination of the propensity score model was assessed using the area under the ROC curve [21], with higher values indicating better discrimination. Calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test [20]. The Hosmer-Lemeshow test compares model performance (observed *versus* expected) across deciles of risk to test whether the model is biased (*i.e.*, performs differently at the extremes of risk). A non-significant value for the Hosmer-Lemeshow test suggests an absence of such bias.

We considered p-values of less than 0.05 to be statistically significant.

Statistical analysis was performed with SPSS, version 18.0 for Windows (IBM Japan, Tokyo, Japan).

Results

Overall patient characteristics

Table 2 shows the baseline characteristics of all 315 patients before propensity score matching. In the BCAA and non-BCAA groups, there were significant differences in HCC etiology, interferon (IFN)-based therapy outcomes in patients infected with hepatitis C virus (HCV), serum albumin levels, prothrombin time, platelet count, and BTR. Regarding treatment for HCC, hepatic resection was the majority in both groups.

Survival due to liver-related mortality in patients not on BCAA therapy (Cohort 1)

The respective three-year, five-year, and seven-year cumulative survival rates associated with liver-related mortality were 82.7%, 73.2%, and 67.4% in patients not on BCAA therapy. Multivariate analysis with Cox proportional hazards modeling using the covariates of age (<65 years or ≥65 years), sex (female or male), BTR (≤4.4 or >4.4), Child-Pugh class (A or B), platelet count (<100 $\times 10^3/\mu l$ or ≥100 $\times 10^3/\mu l$), AFP (<100 ng/ml or ≥100 ng/ml), HCC stage (I or ≥II), and initial HCC treatment (resection or other treatment) showed that low BTR (≤4.4) was an independent factor associated with

poor patient survival from liver-related mortality (hazard ratio [HR], 1.880; 95% CI, 1.125–3.143; p=0.016). In addition, age ≥65 years (HR, 1.949; 95% CI, 1.080–3.515; p=0.027), AFP ≥ 100 ng/ml (HR, 1.902; 95% CI, 1.130–3.202; p=0.015), and HCC stage ≥II (HR, 1.793; 95% CI, 1.057–3.041; p=0.030) were also significantly associated with poor survival in patients after initial treatment for HCC (Table 3).

Patient characteristics after propensity score matching (Cohort 2)

The baseline characteristics of the 132 study patients after propensity score matching are summarized in Table 4. In the BCAA and non-BCAA groups (controls), there were no significant differences except for INF-based therapy outcomes in patients with HCV. The duration of BCAA granule therapy was 1.4 years (range, 0.3–9.4).

Survival from liver-related mortality in patients after propensity score matching (Cohort 2)

The respective three-year, five-year, and seven-year cumulative survival rates from liver-related mortality were 83.7%, 69.5%, and 60.8% in patients after propensity score matching. Multivariate analysis with Cox proportional hazards modeling using the

covariates of age (<65 years or ≥65 years), sex (female or male), BTR (≤4.4 or >4.4), Child-Pugh class (A or B), platelet count (<100 $\times 10^3/\mu l$ or ≥100 $\times 10^3/\mu l$), AFP (<100 ng/ml or ≥100 ng/ml), HCC stage (I or ≥II), initial HCC treatment (resection or other), and BCAA administration status (BCAA group or non-BCAA group) showed that BCAA granule therapy was independently associated with survival from liver-related mortality (HR, 0.524; 95% CI, 0.282–0.973; p=0.041), whereas age ≥65 years (HR, 2.938; 95% CI, 1.386–6.230; p=0.005), and male sex (HR, 2.734; 95% CI, 1.289–5.797; p=0.009) were significantly associated with poor survival (Table 5). BTR was not selected as an independent factor associated with survival in patients after initial treatment for HCC after propensity score matching.

Rates of survival from liver-related mortality based on variables that showed significant differences in the multivariate analysis (Cohort 2)

Cumulative survival rates due to liver-related mortality based on BCAA administration status are shown in Figure 2a. Cumulative survival rates associated with liver-related mortality in patients in the BCAA group were significantly higher than those of patients in the non-BCAA group (p=0.041). The three-year, five-year, and seven-year cumulative survival rates associated with liver-related mortality in the

BCAA group were 91.8%, 73.4%, and 64.2%, respectively, compared to 75.9%, 66.4%, and 57.9%, respectively, in the non-BCAA group.

Cumulative survival rates associated with liver-related mortality by age are shown in Figure 2b. Cumulative survival rates associated with liver-related mortality were significantly lower in patients ≥65 years compared to patients <65 years (p=0.036). The three-year, five-year, and seven-years cumulative survival rates associated with liver-related mortality in patients ≥65 years were 79.5%, 62.9%, and 55.0%, respectively, and those of patients <65 years were 94.3%, 84.1%, and 73.6%, respectively.

Cumulative survival rates associated with liver-related mortality by sex are shown in Figure 2c. There were no differences in survival between males and females (p=0.119). The three-year, five-year, and seven-year cumulative survival rates associated with liver-related mortality in male patients were 82.1%, 63.7%, and 51.5%, respectively, and those of female patients were 87.0%, 82.4%, and 82.4%, respectively.