# IMPORTANCE OF BRANCHED-CHAIN AMINO ACIDS IN PATIENTS WITH LIVER CIRRHOSIS AND ADVANCED HEPATOCELLULAR CARCINOMA RECEIVING HEPATIC ARTERIAL INFUSION CHEMOTHERAPY

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BCAA for HCC patients receiving HAIC.

**Key words:**

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**ABSTRACT**

**PURPOSE:** The aim of this retrospective cohort study was clarify the effect of a branched-chain amino acids (BCAA) on the liver function and the prognosis of Child-Pugh class (C-P) A and B liver cirrhosis (LC) patients with advanced hepatocellular carcinoma (aHCC) undergoing hepatic arterial infusion chemotherapy (HAIC).

**METHODS:** Ninety-two adult Japanese patients with LC and aHCC underwent HAIC. They were in C-P A or B, and they showed multiple partial responses or stable disease. We excluded 11 patients classified as C-P C and 47 patients who showed no response. The patients were divided into an HAIC group receiving HAIC alone (n = 43) and a BCAA group treated with HAIC plus BCAA (n = 49). HAIC was delivered via the proper hepatic artery. The BCAA group also received oral administration of BCAA.

**RESULTS:** In the BCAA group, serum albumin increased significantly after HAIC, while there were no significant changes of serum total bilirubin, serum aminotransferases, prothrombin time, ascites, and hepatic encephalopathy. The C-P score decreased significantly after HAIC compared with before HAIC in C-P B patients, although there was no significant change in C-P A patients. Survival of the BCAA group was significantly longer than that of the HAIC group, with the median survival time being 426 versus 272 days for C-P B patients, although there was no significant difference for C-P A patients.

**CONCLUSIONS:** BCAA might improve the survival and C-P score by increasing serum albumin in C-P B patients with aHCC receiving HAIC.

## I. INTRODUCTION

Sorafenib is an oral multikinase inhibitor that shows strong in vitro activity by targeting the Raf/mitogen-activated protein kinase/extracellular signal-related kinase signaling pathway, and it is used to treat advanced hepatocellular carcinoma (aHCC). In the Sorafenib HCC Assessment Randomised Protocol (SHARP) study, 602 patients (mainly Europeans) were randomized to receive sorafenib therapy or placebo therapy. They had an Eastern Cooperative Oncology Group performance status of 0 - 2 and were all in Child-Pugh (C-P) class A. The sorafenib group achieved a median overall survival time of 10.7 months versus 7.9 months for the placebo group [1]. Sorafenib has also demonstrated significant clinical activity against HCC in phase II and phase III studies [2, 3], with sorafenib-treated patients having a longer median survival time and longer time to radiologic progression compared with placebo-treated patients. However, Pinter et al. reported that complications of sorafenib therapy may occur more frequently among patients in C-P classes B or C [4]. In the most recent Japan Society of Hepatology treatment algorithm for HCC (2010 update), transcatheter arterial chemoembolization (TACE) (for HCC with Vp1 or Vp2) and hepatic arterial infusion chemotherapy (HAIC) are recommended for liver cirrhosis (LC) patients (C-P classes A and B) with four or more tumors, thrombus involving major portal vein branches, or portal trunk thrombus, while sorafenib is only recommended for patients in C-P class A [5]. Therefore, HAIC is still one of the few remaining options for treating aHCC in patients with LC who are in C-P class A or B, as well as LC patients with thrombus involving major branches of the portal vein or the portal trunk. Improvement of implantable drug delivery systems has made it possible to perform repeated hepatic

arterial infusion of anticancer agents in patients with aHCC, and HAIC has been demonstrated to improve both survival and the quality of life [6]. HAIC with 5-fluorouracil (5-FU) and cisplatin (CDDP) using an infuser pump and an implanted reservoir has been related to prolong the survival of aHCC patients [6-8]. We have also reported that combined intra-arterial therapy with low-dose 5-FU, CDDP, and leucovorin (LV) prolongs the survival of aHCC patients [9]. Moreover, we have shown that continuous intra-arterial infusion for 24 hr is more effective than infusion for 6 hr in patients with HCV-related LC and aHCC, although 24-hr infusion also causes stronger hematologic toxicity [10]. Even when dietary intake is adequate, deficiency of branched-chain amino acids (BCAA) can occur due to enhanced uptake and consumption of these amino acids by skeletal muscle for ammonia metabolism and energy generation [11, 12]. Supplementation of BCAA has been reported to improve the nutritional status and prevent hepatic complications in patients with decompensated cirrhosis [13-15]. In addition, Hayashi et al. reported that BCAA supplementation was associated with a lower incidence of HCC in cirrhosis patients and appeared to prevent liver-related events in C-P class A patients [16]. Furthermore, Harima et al. reported that a BCAA-enriched late evening snack improves energy metabolism and glucose tolerance in cirrhosis patients with advanced HCC undergoing HAIC [17]. Therefore, we investigated the effect of BCAA on liver function and the prognosis of LC patients (C-P classes A and B) with aHCC undergoing HAIC in the present retrospective cohort study.

## II. METHODS

### *Patients*

Review of the medical records identified 92 adult Japanese LC patients with LC and aHCC who were managed at Toho University Medical Center Omori Hospital between 2000 and 2011, and who were not eligible for surgical resection or for interventions such as transcatheter arterial embolization, percutaneous ethanol injection, microwave coagulation therapy, or radiofrequency ablation because they had multiple tumors in both lobes of the liver. All 92 patients received HAIC. These patients were all in C-P classes A or B, and they showed multiple partial responses or stable disease. We excluded 11 patients were classified as C-P class C and 47 patients (19 patients were classified as C-P class A and 28 patients were classified as C-P class B) who showed no response because of liver damage caused by tumor progression. The patients were divided into two groups, which were the HAIC group treated by HAIC alone (n = 43) and the BCAA group treated by HAIC plus BCAA (n = 49). HAIC was delivered via the proper hepatic artery at 5-day intervals for 4 weeks. The BCAA group also received oral administration of BCAA (12 g/day of Livact Granules; Ajinomoto Co., Inc., Tokyo, Japan or 3 packets/day of Aminoreban EN powder mix; Otsuka Co., Inc., Tokyo, Japan). Livact Granules contain 952 mg of L-isoleucine, 1904 mg of L-leucine and 1144 mg of L-valine per packet, while one packet of Aminoreban EN contains as follows 1922, 2037, and 1602 mg, respectively. Patients were administered 1 packet of Livact Granules or Aminoreban EN three times a day after meals from the first therapy of HAIC. Blood samples were collected from the patients in the morning before and after chemotherapy.

***Drug delivery system***

In all patients, an intra-arterial catheter was inserted via the femoral artery and was attached to a subcutaneously implanted reservoir [18]. In principle, the gastroduodenal artery and the right gastric artery were occluded with steel coils to prevent gastroduodenal injury by the anticancer agents. Informed consent to these procedures was obtained from all of the patients.

***Treatment***

All patients were treated by 24-hrs HAIC (LV at 12 mg / hr, CDDP at 10 mg / hr, and 5-FU at 250 mg / $m^2$ / 22 hrs) via the proper hepatic artery at 5-day intervals for 4 weeks using a catheter connected to a subcutaneously implanted drug reservoir. The doses of these chemotherapy agents were set according to a previous report [19], and treatment was continued to enforce as much as possible every 3 or 4 months at the same intensity.

***Evaluation of Efficacy***

On CT scans obtained after 4 weeks of treatment, the 2 longest perpendicular diameters of the largest tumor were measured and the product was calculated. A complete response (CR) was defined as disappearance of this tumor (decrease of the product to 0), while a partial response (PR) was defined as reduction of the product by more than 50%. An increase of more than 25% was defined as progressive disease (PD), and smaller changes between PR and PD were defined as stable disease (SD).

### ***Statistical Analysis***

Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS version 11.0; SPSS, Chicago, IL, USA). Results are expressed as the mean ± standard deviation (SD). The $\chi^2$ test was employed to assess differences of patient distribution. Normally distributed variables were compared between the two groups with Student's test and other variables were compared by the Mann-Whitney U test, while Wilcoxon's signed rank sum test was used to compare patient characteristics within the same group. Survival was evaluated by the Kaplan-Meier method, and the significance of differences in survival was determined by the log-rank test. The Cox proportional hazards model was employed for multivariate analyses of factors associated with survival. A probability of less than 0.05 was considered to indicate statistical significance in all analyses.

## III. RESULTS

The HAIC group (n = 43) included 34 men and 9 women with LC who were treated for aHCC by HAIC at our hospital between 2000 and 2011, while the BCAA group (n = 49) comprised 43 men and 6 women with LC who were treated for aHCC by HAIC during the same period and also received BCAA. In the HAIC group, 30 patients were in C-P class A and 13 patients were in class B, while the respective numbers were 23 and 26 in the BCAA group. There was a significant difference in the severity of liver damage between the two groups ($p$ = 0.028). In the HAIC group, 14 patients had stage III disease, 21 patients had stage IVA disease, and 8 patients had stage IVB disease,

while the respective numbers were 8, 36, and 5 in the BCAA group. There was a significant difference of tumor stage between the two groups ($p$ = 0.039). In the HAIC group, 22 patients achieved PR and 21 patients showed SD, while 15 patients showed PR and 34 patients had SD in the BCAA group. There was a significant difference of the tumor response between the two groups ($p$ = 0.046). Moreover, there was a significant difference of the serum albumin level between the HAIC and BCAA groups ($p$ = 0.006) (Table 1). We calculated propensity scores to identify factors with an influence on the survival of the subjects. After excluding serum albumin, the propensity score for treatment with or without BCAA showed < 0.001 based on the Humster-Lemeshow test and the area under the curve (AUC) of the propensity score was 34.2338 (95% confidence interval [CI], 7.0966-175.9901). When serum albumin was included, the propensity score for BCAA still had a significant influence, with $p$ < was 0.0002 and an AUC of 17.2676 (95% confidence interval [CI], 3.8766-81.5265) (Table 2). These findings indicated that treatment with BCAA might improve the survival of LC patients with aHCC undergoing HAIC. Therefore, we separately investigated patients in C-P classes A and B to more clearly define the influence of BCAA on survival after HAIC for aHCC.

***Analysis of patients in Child-Pugh class A***

Table 3 shows the characteristics of the 53 LC patients with advanced HCC classified as C-P class A. The C-P class A patients of the HAIC group and the BCAA group showed no significant differences of gender, age, etiology of cirrhosis, stage, response, $NH_3$, total birilubin (T-Bil), direct birilubin (D-Bil), alanine aminotransferase (ALT), total

cholesterol, white blood cell count, platelet count, prothrombin time (PT), creatinine (Cr), alfa-fetoprotein (AFP), AFP-L3, and des-gamma carboxyprothrombin (DCP). Serum albumin was significantly higher in the HAIC group than in the BCAA group ($p$ = 0.009).

***Multivariate analysis of factors associated with the survival of patients in Child-Pugh class A***

Univariate analysis of factors associated with survival revealed a significant influence of $NH_3$ (hazard ratio [HR], 1.0184, $p$ = 0.008), AFP (HR, 1.0000, $p$ = 0.019), and AFP-L3 (HR, 1.0215, $p$ = 0.007). However, there was no significant difference in relation to BCAA supplementation (HR, 1.3325, $p$ = 0.374) (Table 4). On multivariate analysis with Cox proportional hazards model, BCAA supplementation was also not significantly associated with the survival time of C-P class A patients (HR, 1.4804; 95% CI, 0.7365-3.07580; $p$ = 0.273), while tumor stage (HR, 2.81397; 95% CI, 1.29716-6.53700; $p$ = 0.008 ) and AFP (HR, 2.2080; 95% CI, 1.0056-5.3843; $p$ = 0.048) had a significant influence on the survival time (Table 5).

***Analysis of patients in Child-Pugh class B***

Table 6 shows the characteristics of the 39 patients with advanced HCC in C-P class B. Ptients in the HAIC group and the BCAA group showed no significant differences of gender, etiology of cirrhosis, stage, response, $NH_3$, T-Bil, D-Bil, albumin, ALT, total cholesterol, WBC count, PLT count, PT, AFP, AFP-L3, and DCP. The mean age was significantly higher in the HAIC group than in the BCAA group ($p$ = 0.028).

***Multivariate analysis of factors associated with the survival of patients in Child-Pugh class B***

Univariate analysis of factors associated with survival revealed a significant influence of $NH_3$ (hazard retio [HR], 0.9742, $p = 0.030$) and BCAA supplementation (HR, 2.3018, $p = 0.039$) (Table 7). Multivariate analysis with the Cox proportional hazards model confirmed that BCAA supplementation was associated with the survival time of C-P class B receiving HAIC for aHCC (HR, 2.7889; 95% CI, 1.2247-6.3213; $p = 0.015$), while tumor stage (HR, 1.9823; 95% CI, 0.5536-12.7597; $p = 0.328$ ) and AFP (HR, 2.1735; 95% CI, 0.8776-6.3353; $p = 0.097$) were not determinants of the survival time (Table 8).

***Serum alanine aminotransferase***

Figure 1 summarizes the changes of ALT in each group. In the HAIC group, there were no significant changes of serum ALT after chemotherapy among patients in either C-P class A (before: 46.0 $\pm$ 20 IU/l, after: 45.7 $\pm$ 47 IU/l) or class B (before: 48.3 $\pm$ 37 IU/l, after: 45.0 $\pm$ 38 IU/l). In the BCAA group, there were also no significant changes of serum ALT after chemotherapy among patients in C-P class A (before: 46.7 $\pm$ 47 IU/l, after: 37.6 $\pm$ 37 IU/l) or class B (before: 51.5 $\pm$ 45 IU/l, after: 49.4 $\pm$ 47 IU/l).

***Serum albumin***

Figure 2 summarizes the changes of serum albumin in each group. In the HAIC group, there were no significant changes of serum albumin after chemotherapy among

the patients in C-P class A (before: 3.58 ± 0.4, after: 3.66 ± 0.4 g/dl) or those in class B (before: 2.81 ± 0.6, after: 2.99 ± 0.6 g/dl). In the BCAA group, however, serum albumin showed a significant increase after chemotherapy compared with before chemotherapy for patients in C-P class B (before:2.85 ± 0.4, after: 3.09 ± 0.5 g/dl, $p = 0.009$), while there was no significant change of albumin after chemotherapy in C-P class A patients (before: 3.29 ± 0.3, after: 3.51 ± 0.4 g/dl).

***Total bilirubin***

Figure 3 summarizes the changes of serum T-Bil. In the HAIC group, there was no significant change of serum T-Bil after chemotherapy among patients in C-P class A (before: 0.79 ± 0.4 IU/l, after: 0.76 ± 0.3 IU/l) or those in class B (before: 1.15 ± 0.5 IU/l, after: 1.21 ± 0.5 IU/l). In the BCAA group, there was also no significant change of serum T-Bil after chemotherapy among patients in C-P class A (before: 0.82 ± 0.3 IU/l, after: 0.81 ± 0.3 IU/l) or patients in class B (before: 1.16 ± 0.4 IU/l, after: 1.28 ± 0.4 IU/l).

***Prothrombin time***

Figure 4 summarizes the %PT data. There was no significant change of %PT after chemotherapy among patients from the HAIC group in C-P class A (before: 87.1 ± 14 %, after: 83.7 ± 10 %) or class B (before: 74.9 ± 15 %, after: 74.4 ± 16 %). Likewise, there was no significant change of %PT after chemotherapy among patients from the BCAA group in C-P class A (before: 84.8 ± 11 %, after: 82.9 ± 11 %) or class B (before: 75.5 ± 11%, after: 74.8 ± 13 %).

***Child-Pugh score***

Figure 5 compares C-P scores. In the HAIC group, there was no significant difference of the C-P score between before and after chemotherapy in class A or class B patients (C-P class A: 5.53 ± 0.5 vs. 5.50 ± 0.6, C-P class B: 7.30 ± 1.3 vs. 7.08 ± 1.3). In the BCAA group, however, the C-P score showed a significant decrease after chemotherapy compared with before chemotherapy for patients in C-P class B (7.12 ± 1.0 vs. 7.44 ± 0.7, $p = 0.011$), while there was no significant difference between before and after chemotherapy for C-P class A patients (before: 5.58 ± 0.5, after: 5.46 ± 0.7).

***Survival***

Among patients in C-P class A from the two groups, there was no significant difference of the median survival time (HAIC group: 626 ± 29 days vs. BCAA group: 653 ± 127 days) (Fig. 6). Among patients in C-P class B, however, the median survival time of those from the BCAA group (426 ± 30 days) was significantly longer than that of those from the HAIC group (272 ± 65 days) ($p = 0.0294$, Kaplan-Meier method and log-rank test)(relative hazard ratio: 1.36, 95% confidence interval: 1.06-4.98) (Fig. 7).

## IV. DISCUSSION

The liver plays an important role in energy metabolism, and liver diseases can lead to abnormalities of nutrient metabolism and malnutrition [20]. Among attempts to improve such malnutrition, intake of a late evening snack (LES) by patients with LC has been demonstrated to be beneficial for energy substrate metabolism [21-24]. It has also

been reported that an LES containing BCAA improves malnutrition, amino acid imbalances, and glucose intolerance in patients with LC [25-27]. In present study, an LES was not used and BCAA were administered three times a day after meals. In addition, whether BCAA supplementation improves energy metabolism was not determined. Harima et al. reported that an LES containing BCAA improves energy metabolism and glucose tolerance in LC patients with aHCC undergoing HAIC [17]. However, they did not discuss whether BCAA had an influence on the prognosis of their patients and did not investigate any patients in C-P class B. In the present study, we not only assessed LC patients in C-P class A with aHCC undergoing HAIC, but also those in C-P class B. We investigated who showed multiple partial responses or had stable disease and we excluded patients in C-P class C and patients with no response. Because liver damage caused by tumor progression should influence the prognosis of LC patients with aHCC undergoing HAIC, we calculated propensity scores to find other factors with an influence on survival. Our results showed that treatment with BCAA might improve the survival time of aHCC patients undergoing HAIC. We also investigated in C-P classes A and B separately to confirm that treatment with BCAA was associated with the survival of these patients. Multivariate analysis with the Cox proportional hazards model showed that BCAA supplementation was not associated with the survival time of aHCC patients in C-P class A undergoing HAIC, while tumor stage and AFP were associated with their survival. However, BCAA supplementation was associated with the survival time of aHCC patients in C-P class B receiving HAIC, whereas tumor stage and AFP did not influence the survival of these patients. In C-P class B patients from the BCAA group, serum albumin was significantly increased after

HAIC, while there was no significant change in serum T-Bil, serum ALT, %PT, ascites, or hepatic encephalopathy. Therefore, the C-P score showed a significant decrease after HAIC compared with before HAIC in C-P class B patients from this group, although there was no significant change in C-P class A patients from the same group. Moreover, the survival time of C-P class B patients from the BCAA group was significantly longer than that of class B patients from the HAIC group, while the median survival time showed no significant difference for C-P class A patients from the 2 groups. The increasing of serum albumin by BCAA treatment might have improved C-P score and might have extended the period which can be treated by HAIC. These might contribute it to extension of survival time. Vincent et al. reported a decreased frequency of LC complications and an improved nutritional status when BCAA were prescribed as maintenance therapy for LC patients [28]. They also reported that nutritional supplementation with oral BCAA is beneficial for increasing the serum albumin level, reducing morbidity, and improving quality of life in patients undergoing TACE for HCC [29]. In the present study, BCAA improved the survival time and C-P score by increasing serum albumin in C-P class B patients receiving HAIC for aHCC. These findings might have reflected improvement of energy metabolism and glucose tolerance in our LC patients with advanced HCC undergoing HAIC, although we did not investigate nutritional parameters.

BCAA (LIVACT granules) were reported to improve the plasma albumin level in LC patients with hypoalbuminemia [30, 31]. A BCAA-enriched snack (Aminoreban EN powder mix) was superior to ordinary food as a late evening snack for improving the serum albumin level and energy metabolism of LC patients [24]. Ijichi et al. reported

that BCAA, especially leucine, promote the production of albumin by rat hepatocytes via the mammalian target of rapamycin (mTOR) signal transduction system [32]. Furthermore, it has been reported that oral administration of BCAA augments hepatic albumin synthesis, not only by supplementation of substrates for protein synthesis, but also by induction of mTOR signaling that is critical for initiation of translation, and that activation of the mTOR pathway is one of the major mechanisms by which BCAA treatment reverse hypoalbuminemia in LC patients [33].

In conclusion, the present study showed that BCAA treatment can improve the liver function and prognosis of LC patients in C-P class B undergoing HAIC for aHCC. It was suggested that BCAA might increase the serum albumin level by promoting hepatic albumin production and might thus allow the prolongation of HAIC. These effects might improve the prognosis of LC patients with aHCC in C-P class B.

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**Figure legends**

Figure 1. Changes of serum alanine aminotransferases (ALT) in the two groups. In the HAIC group, there were no significant changes of serum ALT after chemotherapy among patients in Child-Pugh class A or class B. In the BCAA group, there were also no significant changes of serum ALT after

chemotherapy among patients in Child-Pugh class A or class B.

Figure 2. Changes of serum albumin in the two groups. In the HAIC group, there were no significant changes of serum albumin after chemotherapy among patients in Child-Pugh class A or class B. In the BCAA group, serum albumin was significantly increased after chemotherapy compared with before chemotherapy for patients in Child-Pugh class B, while there was no significant difference of serum albumin between before and after chemotherapy for Child-Pugh class A patients.

Figure 3. Changes of serum total total birilubin (T-Bil) in the two groups. In the HAIC group, there were no significant changes of serum T-Bil after chemotherapy among patients in Child-Pugh class A or class B. In the BCAA group, there were also no significant changes of serum T-Bil after chemotherapy among patients in Child-Pugh class A or class B.

Figure 4. Changes of the prothrombin time (%PT) in the two groups. In the HAIC group, there were no significant changes of the %PT after chemotherapy among patients in Child-Pugh class A or class B. In the BCAA group, there were also no significant changes of the %PT after chemotherapy among patients in Child-Pugh class A or class B.

Figure 5. Changes of the Child-Pugh score in the two groups. In the HAIC group,

there was no significant difference of the Child-Pugh score between before and after chemotherapy. In the BCAA group, however, the Child-Pugh score was significantly lower after chemotherapy compared with before chemotherapy for patients in Child-Pugh class B, although there was no significant difference between before and after chemotherapy in Child-Pugh class A patients.

Figure 6. Survival time of patients with advanced hepatocellular carcinoma from Child-Pugh class A in each group. There was no significant difference of the median survival of class A patients between the two groups.

Figure 7. Survival time of patients with advanced hepatocellular carcinoma in Child-Pugh class B from each group. The median survival time of class B patients from the BCAA group was significantly longer than that of those from the HAIC group.

**Table 1.**
**Comparison of clinical characteristics between the BCAA and HAIC groups**

| | BCAA group | HAIC group | *P value* |
|---|---|---|---|
| No. of patients | 49 | 43 | |
| Mean age | 66.3 ± 7 | 68.0 ± 7 | *0.209* |
| Gender (M/F) | 43/6 | 34/9 | *0.283* |
| Etiology of cirrhosis (HBV/HCV/non Bnon C) | 8/30/11 | 6/30/7 | *0.745* |
| Child-Pugh classification (A/B) | 23/26 | 30/13 | *0.028** |
| Stage (IV/III) | 41/8 | 29/14 | *0.070* |
| Stage (III/IVA/IVB) | 8/36/5 | 14/21/8 | *0.039** |
| Response(PR/SD) | 15/34 | 22/21 | *0.046** |
| $NH_3$ (μg/dl) | 67.5 ± 40 | 56.3 ± 30 | *0.268* |
| total birilubin (g/dl) | 1.03 ± 0.4 | 0.92 ± 0.5 | *0.236* |
| direct birilubin (g/dl) | 0.34 ± 0.2 | 0.31 ± 0.3 | *0.495* |
| albumin (mg/dl) | 3.04 ± 0.4 | 3.33 ± 0.5 | *0.006** |
| ALT (IU/l) | 50.0 ± 45 | 44.5 ± 20 | *0.473* |
| total cholesterol (mg/dl) | 152.0 ± 56 | 157.0 ± 66 | *0.758* |
| white blood cell (/$mm^3$) | 4057 ± 1661 | 4714 ± 2509 | *0.154* |
| platelets (X $10^4$/$mm^3$) | 11.76 ± 6.8 | 14.52 ± 8.0 | *0.087* |
| prothrombin time (%) | 80.0 ± 12 | 84.8 ± 14 | *0.083* |
| BUN (mg/dl) | 13.9 ± 6 | 13.4 ± 6 | *0.067* |
| Cr (mg/dl) | 0.76 ± 0.3 | 0.69 ± 0.2 | *0.131* |
| AFP (ng/ml) | 15260 ± 55227 | 36790 ± 161779 | *0.412* |
| AFP (<20 / ≥20 ng/ml) | 9/34 | 9/32 | *0.909* |
| AFP-L3 (%) | 35.6 ± 26 | 26.9 ± 25 | *0.163* |
| DCP (AU/ml) | 75263 ± 330127 | 9712 ± 26423 | *0.226* |

HBV: hepatitis B virus, HCV: hepatitis C virus, $NH_3$: ammonia, ALT: alanine aminotransferase, BUN: blood urea nitrogen, Cr: creatine, AFP: alpha-fetoprotein, DCP: Des-gamma carboxyprothrombin

*Statistically difference compared between BCAA group and HAIC group (the Mann-Whitney U test)

**Table 2.**
**Propensity scores of factors related to the survival of 92 advanced HCC patients treated by HAIC**

**Excluding albumin**

| | Hazard ratio | 95% confidence interval | |
|---|---|---|---|
| | | Lower limit - upper limit | *P value* |
| with or without BCAA | 0.4816 | 0.2705 - 0.8537 | *0.0124** |
| propensity score | 34.2338 | 7.0966 - 175.9901 | *< 0.001*** |

**Including albumin**

| | Hazard ratio | 95% confidence interval | |
|---|---|---|---|
| | | Lower limit - upper limit | *P value* |
| with or without BCAA | 0.5567 | 0.3203 - 0.9682 | *0.0038** |
| propensity score | 17.2676 | 3.8766 - 81.5265 | *< 0.0002*** |

*Statistically difference compared by the Cox proportional hazards model for multivariate analyses of factors associated with survival.

**Statistically difference compared by the propensity score for treatment with or without BCAA showed < 0.001 based on the Humster-Lemeshow test and the area under the curve (AUC) of the propensity score.

**Table 3.**
**Comparison of clinical characteristics for 53 patients in Child-Pugh class A**

| | BCAA group | HAIC group | *P value* |
|---|---|---|---|
| No. of patients | 23 | 30 | |
| Mean age | 66.8 ± 8 | 66.7 ± 7 | *0.644* |
| Gender (M/F) | 20/3 | 23/7 | *0.343* |
| Etiology of cirrhosis (HBV/HCV/non Bnon C) | 3/16/4 | 4/23/3 | *0.730* |
| Stage (IV/III) | 18/5 | 17/13 | *0.100* |
| (IVA/III) | 17/5 | 12/13 | *0.042** |
| (IVB/III) | 1/5 | 5/13 | *0.721* |
| (IVB/IVA) | 1/17 | 5/12 | *0.232* |
| Response(PR/SD) | 8/15 | 16/14 | *0.179* |
| $NH_3$ (μg/dl) | 69.2 ± 54 | 46.8 ± 35 | *0.267* |
| total birilubin (g/dl) | 0.82 ± 0.3 | 0.79 ± 0.4 | *0.781* |
| direct birilubin (g/dl) | 0.27 ± 0.2 | 0.22 ± 0.2 | *0.456* |
| albumin (mg/dl) | 3.29 ± 0.3 | 3.58 ± 0.4 | *0.009** |
| (<3.5 / ≥3.5 mg/dl) | (13/6) | (10/18) | *0.028** |
| ALT (IU/l) | 47.8 ± 48 | 46.0 ± 20 | *0.858* |
| total cholesterol (mg/dl) | 152.4 ± 48 | 155.3 ± 35 | *0.815* |
| white blood cell (/$mm^3$) | 4044.4 ± 1632 | 4964.3 ± 2832 | *0.219* |
| platelets (X $10^4$/$mm^3$) | 12.4 ± 8 | 15.0 ± 8 | *0.302* |
| prothrombin time (%) | 85.4 ± 11 | 87.1 ± 14 | *0.654* |
| BUN (mg/dl) | 14.7 ± 7 | 13.1 ± 5 | *0.366* |
| Cr (mg/dl) | 0.81 ± 0.3 | 0.66 ± 0.2 | *0.044* |
| AFP (ng/ml) | 6400.8 ± 22723 | 2019.2 ± 6278 | *0.347* |
| AFP (<20 / ≥20 ng/ml) | (3/14) | (7/20) | *0.523* |
| AFP-L3 (%) | 30.6 ± 27 | 27.6 ± 26 | *0.738* |
| DCP (AU/ml) | 131040.1 ± 503988 | 3517.5 ± 10439 | *0.220* |

HBV: hepatitis B virus, HCV: hepatitis C virus, $NH_3$: ammonia, ALT: alanine aminotransferase, BUN: blood urea nitrogen, Cr: creatine, AFP: alpha-fetoprotein, DCP: Des-gamma carboxyprothrombin

*Statistically difference compared between BCAA group and HAIC group (the Mann-Whitney U test).

**Table 4.**
**Univariate analysis of factors related to the survival of 53 patients in Child-Pugh class A**

| Factor | Hazard ratio | *P value* |
|---|---|---|
| Mean age | 0.9930 | *0.721* |
| Gender (M/F) | 0.4861 | *0.080* |
| Etiology of cirrhosis (HBV/HCV/non Bnon C) | | |
| (HBV/nonBnonC) | 0.5147 | *0.339* |
| (HCV/nonBnonC) | 0.7945 | *0.618* |
| Stage (IV/III) | 1.5208 | *0.191* |
| (IVA/III) | 1.3799 | *0.342* |
| (IVB/III) | 2.4235 | *0.094* |
| (IVB/IVA) | 1.7562 | *0.257* |
| Response(PR/SD) | 1.7421 | *0.058* |
| $NH_3$ (μg/dl) | 1.0184 | *0.008** |
| total birilubin (g/dl) | 1.6534 | *0.256* |
| direct birilubin (g/dl) | 2.4874 | *0.223* |
| albumin (mg/dl) | 1.3359 | *0.517* |
| (<3.5 / ≥3.5 mg/dl) | 1.3359 | *0.517* |
| ALT (IU/l) | 1.0112 | *0.107* |
| total cholesterol (mg/dl) | 1.0047 | *0.300* |
| white blood cell (/$mm^3$) | 1.0000 | *0.890* |
| platelets (X $10^4$/$mm^3$) | 1.0000 | *0.801* |
| prothrombin time (%) | 1.0025 | *0.849* |
| BUN (mg/dl) | 0.9884 | *0.701* |
| Cr (mg/dl) | 0.3061 | *0.096* |
| AFP (ng/ml) | 1.0000 | *0.019** |
| AFP (<20 / ≥20 ng/ml) | 1.6020 | *0.226* |
| AFP-L3 (%) | 1.0215 | *0.008** |
| DCP (AU/ml) | 1.0000 | *0.169* |
| with or without BCAA | 1.3325 | *0.374* |

HBV: hepatitis B virus, HCV: hepatitis C virus, $NH_3$: ammonia, ALT: alanine aminotransferase, BUN: blood urea nitrogen, Cr: creatine, AFP: alpha-fetoprotein, DCP: Des-gamma carboxyprothrombin

*Statistically difference compared by the Cox proportional hazards model for univariate analyses of factors associated with survival.

**Table 5.**
**Multivariate analysis of factors related to the survival of 53 patients in Child-Pugh class A**

| Factor | Hazard ratio | 95% confidence interval | |
|---|---|---|---|
| | | Lower limit - upper limit | *P value* |
| Stage (IV/III) | 2.8140 | 1.2972 - 6.5370 | *0.008** |
| AFP (< 20 / ≥ 20 ng/dl) | 2.2080 | 1.0056 - 5.3843 | *0.048** |
| with or without BCAA | 1.4804 | 0.7365 - 3.0758 | *0.273* |

AFP: alpha–fetoprotein

*Statistically difference compared by the Cox proportional hazards model for multivariate analyses of factors associated with survival.

**Table 6.**
**Comparison of clinical characteristics for 39 patients in Child-Pugh class B**

| | BCAA group | HAIC group | *P value* |
|---|---|---|---|
| No. of patients | 26 | 13 | |
| Mean age | 65.8 ± 6 | 70.4 ± 4 | *0.028** |
| Gender (M/F) | 23/3 | 11/2 | *0.735* |
| Etiology of cirrhosis (HBV/HCV/non Bnon C) | 5/14/7 | 2/7/4 | *0.943* |
| Stage (IV/III) | 23/3 | 12/1 | *0.719* |
| (IVA/III) | 19/3 | 9/1 | *0.889* |
| (IVB/III) | 4/3 | 3/1 | *0.648* |
| (IVB/IVA) | 4/19 | 3/9 | *0.159* |
| Response(PR/SD) | 7/19 | 6/7 | *0.230* |
| $NH_3$ (μg/dl) | 66.3 ± 28 | 60.9 ± 20 | *0.582* |
| total birilubin (g/dl) | 1.20 ± 0.4 | 1.20 ± 0.6 | *0.981* |
| direct birilubin (g/dl) | 0.40 ± 0.2 | 0.51 ± 0.4 | *0.202* |
| albumin (mg/dl) | 2.86 ± 0.4 | 2.91 ± 0.4 | *0.731* |
| (<3.5 / ≥3.5 mg/dl) | (25/1) | (13/0) | *0.474* |
| ALT (IU/l) | 51.4 ± 44 | 42.2 ± 19 | *0.480* |
| total cholesterol (mg/dl) | 153.0 ± 62 | 132.5 ± 39 | *0.301* |
| white blood cell ( /$mm^3$) | 4065.4 ± 1714 | 4092.3 ± 1672 | *0.963* |
| platelets ( X $10^4$/$mm^3$) | 11.3 ± 6 | 11.5 ± 4 | *0.874* |
| prothrombin time (%) | 75.9 ± 10 | 78.6 ± 10 | *0.500* |
| BUN (mg/dl) | 12.4 ± 5 | 14.1 ± 7 | *0.725* |
| Cr (mg/dl) | 0.73 ± 0.2 | 0.75 ± 0.2 | *0.848* |
| AFP (ng/ml) | 21052.3 ± 68596 | 34528.4 ± 79444 | *0.586* |
| AFP (<20 / ≥20 ng/ml) | (6/20) | (2/11) | *0.575* |
| AFP-L3 (%) | 39.0 ± 26 | 27.5 ± 26 | *0.236* |
| DCP (AU/ml) | 40938.9 ± 152033 | 10819.2 ± 19052 | *0.484* |

HBV: hepatitis B virus, HCV: hepatitis C virus, $NH_3$: ammonia, ALT: alanine aminotransferase, BUN: blood urea nitrogen, Cr: creatine, AFP: alpha-fetoprotein, DCP: Des-gamma carboxyprothrombin

*Statistically difference compared between BCAA group and HAIC group (the Mann-Whitney U test)

**Table 7.**
**Univariate analysis of factors related to the survival of 39 patients in Child-Pugh class B**

| Factor | Hazard ratio | *P value* |
|---|---|---|
| Mean age | 1.0079 | *0.779* |
| Gender (M/F) | 0.9569 | *0.929* |
| Etiology of cirrhosis (HBV/HCV/non Bnon C) | | |
| (HBV/nonBnonC) | 0.8936 | *0.841* |
| (HCV/nonBnonC) | 1.1923 | *0.675* |
| Stage (IV/III) | 2.0280 | *0.288* |
| (IVA/III) | 1.7541 | *0.416* |
| (IVB/III) | 4.1627 | *0.060* |
| (IVB/IVA) | 2.3732 | *0.095* |
| Response(PR/SD) | 0.7896 | *0.519* |
| $NH_3$ (μg/dl) | 0.9742 | *0.030** |
| total birilubin (g/dl) | 0.5587 | *0.207* |
| direct birilubin (g/dl) | 0.6267 | *0.553* |
| albumin (mg/dl) | 0.7864 | *0.629* |
| (<3.5 / ≥3.5 mg/dl) | 0.3777 | *0.413* |
| ALT (IU/l) | 0.9961 | *0.609* |
| total cholesterol (mg/dl) | 0.9961 | *0.386* |
| white blood cell ( /$mm^3$) | 1.0003 | *0.052* |
| platelets ( X $10^4$/$mm^3$) | 1.0000 | *0.902* |
| prothrombin time (%) | 1.0156 | *0.456* |
| BUN (mg/dl) | 1.0610 | *0.110* |
| Cr (mg/dl) | 1.9773 | *0.553* |
| AFP (ng/ml) | 1.0000 | *0.759* |
| AFP (<20 / ≥20 ng/ml) | 2.0090 | *0.119* |
| AFP-L3 (%) | 1.0117 | *0.179* |
| DCP (AU/ml) | 1.0000 | *0.057* |
| with or without BCAA | 2.3018 | *0.039** |

HBV: hepatitis B virus, HCV: hepatitis C virus, $NH_3$: ammonia, ALT: alanine aminotransferase, BUN: blood urea nitrogen, Cr: creatine, AFP: alpha-fetoprotein, DCP: Des-gamma carboxyprothrombin

*Statistically difference compared by the Cox proportional hazards model for univariate analyses of factors associated with survival.

**Table 8.**
**Multivariate analysis of factors related to the survival of 39 patients in Child-Pugh class B**

| Factor | Hazard ratio | 95% confidence interval | |
|---|---|---|---|
| | | Lower limit - upper limit | *P value* |
| Stage (IV/III) | 1.9823 | 0.5536 - 12.7597 | *0.328* |
| AFP (< 20 / ≥ 20 ng/dl) | 2.1735 | 0.8776 - 6.3353 | *0.097* |
| with or without BCAA | 2.7889 | 1.2247 - 6.3213 | *0.015** |

AFP: alpha–fetoprotein

*Statistically difference compared by the Cox proportional hazards model for multivariate analyses of factors associated with survival.