An ABO blood type-identical liver was finally offered and an orthotopic transplantation was performed. The cold ischemic time was 465 min and the graft weight was 1,590 g. The weight of the explanted liver was 836 g. The piggyback procedure was performed, with a blood loss of 16,500 ml. The duration of the operation was approximately 705 min, mainly because of disturbed clotting profile as well as difficulty obtaining complete hemostasis owing to the patient's hemophilic status. Splenectomy was also performed as a result of a low platelet count ($<50{,}000/\mu l$) and possible postoperative need for interferon or other anti-HCV drugs. Owing to the difficulty of achieving complete hemostasis, our strategy was to finally perform gauze packing and removal, on postoperative day (POD) 3. After the depacking, the postoperative course was rather uneventful and without any severe infectious complications.

Histological examination revealed marked variation in the size and shape of the hepatic nodules, known as mixed micro- and macro-nodular cirrhosis. Persistent active inflammation with lymphoid follicles and interface activity was observed in the portal tract and in the thick fibrous septa. There was no evidence of hepatocellular carcinoma. With regards to immunosuppression, we tried to avoid a steroid bolus in order to prevent infectious complications due to the nature of the HIV disease and HCV flares. Anti-CD25 antibody was administered on POD 1 and 4, and tacrolimus was administered on POD 2, followed by aiming the trough level around POD 8 with steroid tapering (Fig. 1). On POD 7, ART was resumed and continued thereafter. The patient's CD4 count was preserved and even elevated owing to the splenectomy. The postoperative course of the patient was uneventful and he was discharged home on day 44 after the DDLT. He was administered the same dose and type of ART as before the DDLT. Anti-HCV treatment with combination of pegylated-interferon, ribavirin, and simeprevir was initiated on day 110 after the DDLT.

However, a sustained viral response was not achieved, and therefore, we initiated treatment with a new direct-acting antiviral agent anti-HCV drugs (DAA), sofosbuvir. The definitive outcomes of the antiviral treatment will be evaluated in the future.

We changed the ART from darunavir/ritonavir to raltegravir before LT for this purpose, which made it possible for us to use regular immunosuppressive agents (8). HIV RNA level has been undetectable throughout the observation period. Before the advent of raltegravir, most ART drugs, non-nucleoside reverse transcriptase inhibitors or protease inhibitors, interacted with immunosuppressive agents such as tacrolimus or cyclosporine because they are all metabolized by the same cytochrome P450 family (CYP3A4). Regarding the CD4

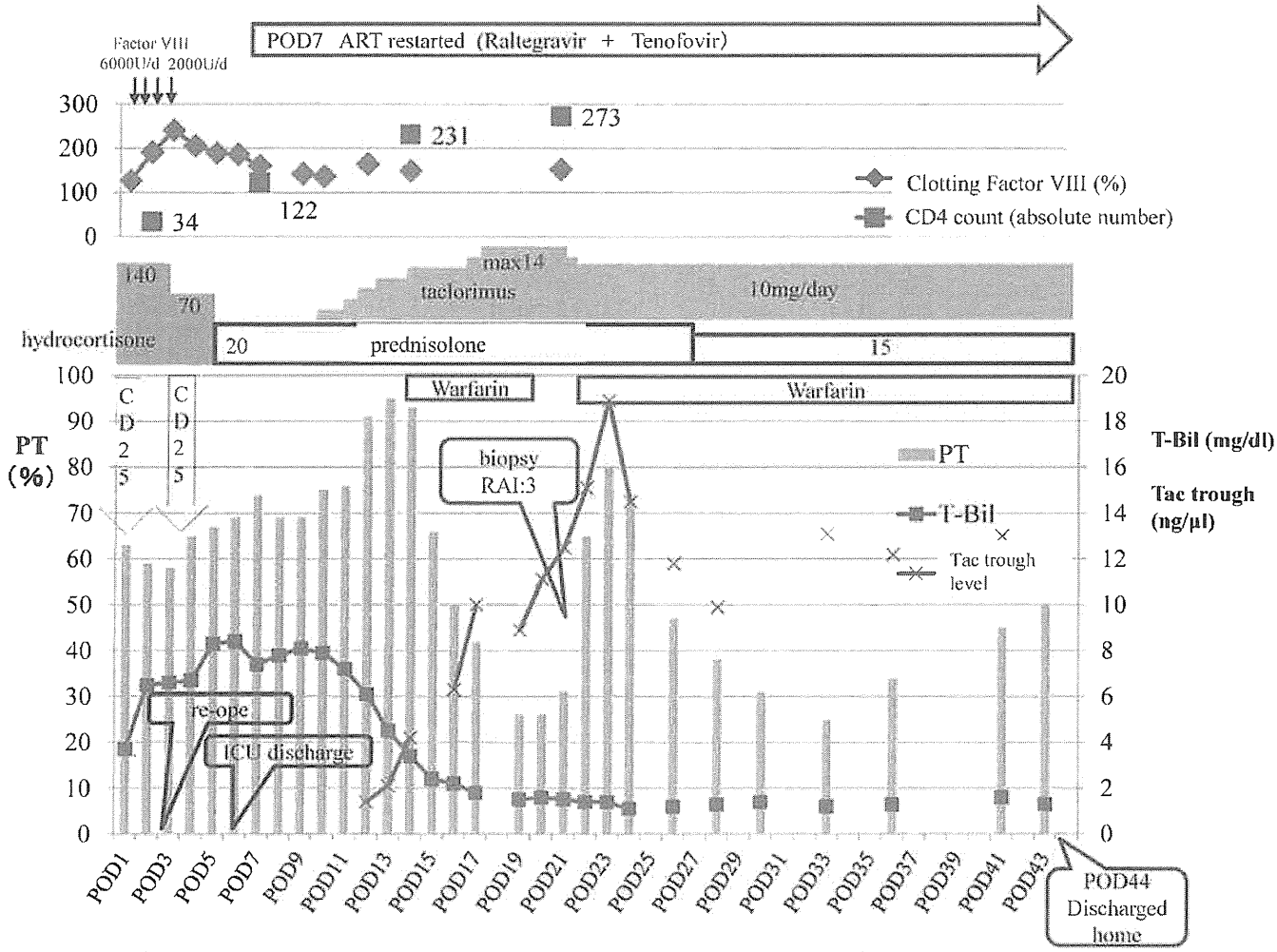


Fig. 1. Post-transplant course in a hemophilic patient with HIV/HCV co-infection. ART restarted (raltegravir, tenofovir) on day 7 and tacloriums on day 11 after the DDLT.

count, our indication for LT was a level above 100, owing to the hypersplenism due to severe portal hypertension. Immediately after the DDLT, the CD4 count dropped below 100, although it recovered spontaneously with the aid of a splenectomy, which was scheduled before the DDLT was performed (9). We believe that if the HIV titer is under control with ART, the absolute CD4 number for indication of LT could be lowered to 100, but not 200 because hypersplenism can mask the real immunological function of those patients with HIV/HCV coinfection. Also, splenectomy may increase the CD4 count and strengthen the immune function before or during LT. According to the article by Nomura et al., after splenectomy, the ratio of CD4 cells in peripheral blood decreases leading to a significant decrease in the CD4/CD8 ratio in patients with liver cirrhosis (10). Therefore, a splenectomy may not significantly increase the CD4 count before LT. However, Hashimoto et al. reported that the CD4 function, in terms of IFN-gamma production and CD4 proliferation, increased after splenectomy (11). Accordingly, it is still controversial whether splenectomy should be performed before LT for patients with HIV infection in order to increase the number or function of CD4 cells.

HCV infection control after DDLT is an important factor because the progression of fibrosis is quicker in patients with HIV/HCV coinfection than in patients with HCV infection only (12–14). In 2014, strong direct acting anti-HCV drugs were more commonly available, and currently in Japan, even HIV/HCV coinfected patients are administered DAAs such as sofosbuvir for better outcomes after DDLT (15). Accumulation of results regarding the use of DAAs in Japanese patients, first for HCV mono-infected patients, is necessary.

After implementation of additional points for HIV/HCV coinfected patients for eligibility for DDLT, it became possible to salvage those coinfected patients in whom the prognosis was definitely worse than that for HCV mono-infected patients, especially for those with platelet counts less than 10,000 cells/$\mu$l (5). In addition, because those patients were infected with HCV in their childhood, they developed cirrhosis in their 30s or 40s, sometimes with hepatocellular carcinoma. Although only a few patients with HIV/HCV coinfection should be indicated for DDLT, this first report offers special information for such cases.

In conclusion, this is the first report of DDLT performed for an HIV/HCV coinfected hemophilic patient. Meticulous management of ART and clotting factors could lead to the success of DDLT in such cases. Post-transplant anti-HCV therapy will be a key factor to preventing hepatitis recurrence and the progression of fibrosis.

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**Conflict of interest** None to declare.

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State of the Art

# 血液製剤によるHIV/HCV重複感染者に対する肝移植
## ―最近わかった諸々のこと―

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## はじめに

Anti-retroviral therapy(ART)によるHIVコントロールの改善により，HIV/HCV重複感染者の死亡の原因として，肝疾患の割合が増加している[1]。その内訳として，C型慢性肝炎そのものによる非代償性肝不全に加え，ARTなど薬剤性肝障害などによる非硬変性門脈圧亢進症(non-cirrhotic portal hypertension：NCPH)の報告が海外よりなされてきている[2)3)]。これらの患者は最終的には肝移植の適応となる可能性があり，その成績の報告もみられるが，おおむねHCV単独感染に対する移植成績と比較して若干低いがacceptableとするものが多い[4)5)]。ほぼ全例にARTが施行されているため，種々の病態が混在し，肝移植の適応とタイミングを困難なものにしている。本稿ではHIV/HCV重複感染患者の肝機能障害およびその根治療法としての肝移植について最新のレビューを行う。

## 血液製剤によるHIV/HCV重複感染の歴史と肝移植

1980年代の輸入血液製剤によるHIV感染は，いわゆる薬害エイズ事件として1996年に国と和解解決し，1997年国立国際医療センター内にエイズ治療・研究開発センター(ACC)が設立された。1990年代の後半より各種ART製剤の開発進歩が目覚ましく，免疫不全であるエイズ発症は少なくなり，相対的にHIVとともに共感染したHCVによる肝硬変，肝癌発症による死亡が増加してきた[1]。また，上記当該患者は血友病のための血液内科，小児科，感染症内科がフォローしていることも多く，肝疾患は見過ごされていることもあるようである。

イギリスでもLancet誌に同様の問題が報告され，

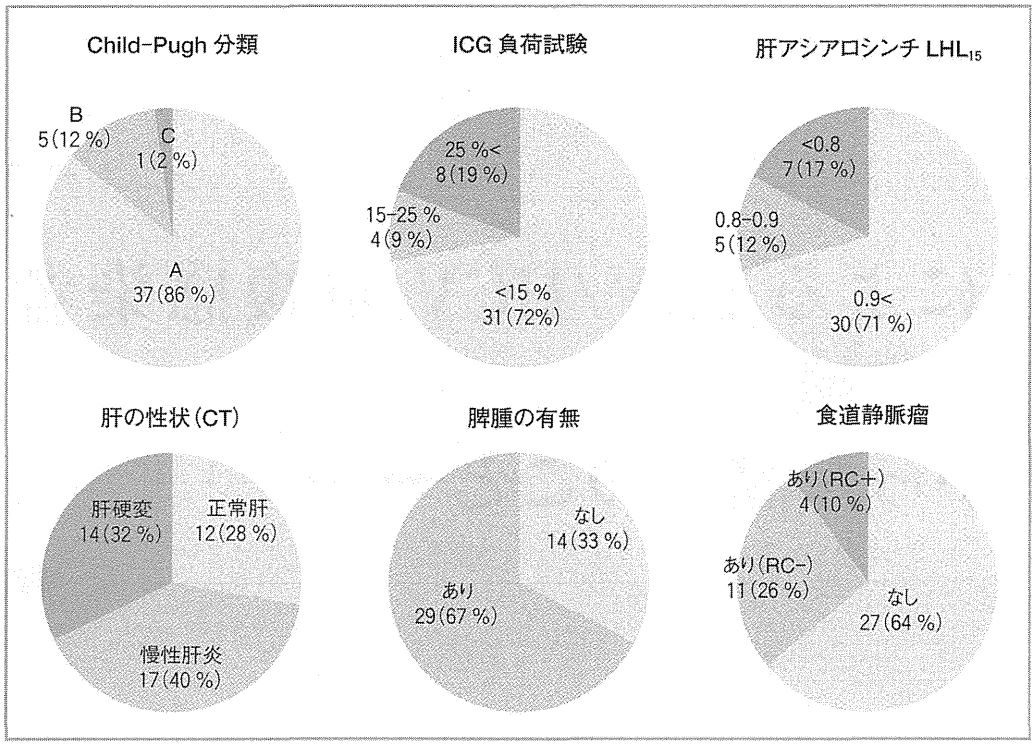


図1　HIV/HCV 重複感染者の肝機能評価(長崎大学での検査施行例のみ)
(文献 11 より引用)

薬害血友病患者における肝硬変での死亡率上昇が問題視された[6]。一方，フランスを中心に欧州では HIV 陽性患者(血友病ではないが)に対する肝移植の報告が増えている[7]。特にイタリアの Bacccarani は HIV/HCV 重複感染者に対する肝移植に積極的に取り組み種々の報告をしており[8)9)]，彼には第 27 回日本エイズ学会(熊本)の特別講演者として来日していただき，本問題についての講演および意見交換を行った。このように世界的にも HIV 感染患者に対する肝移植の報告例は増加してきている。つまり HIV 陽性であっても肝移植禁忌ではないのである。

## 血液製剤による HIV/HCV 重複感染者の肝機能評価と予後

さて，それでは本邦での現況はどうであろうか？平成 25 年度の血液凝固異常症全国調査では HIV/HCV 重複感染血液凝固異常患者での慢性肝炎患者は 353 人，肝硬変患者は 64 人となっている[1]。照屋先生の調査では，肝移植の適応となる Child-Pugh B 以上は 20 人と試算されている[20]。しかし，実際の患者さん，特に通院中の Child-Pugh A の患者さんの肝病態についてははっきりとした報告が存在しなかった。そこでわれわれは血液製剤による HIV/HCV 重複感染者の肝機能検査を施行し，一般肝機能検査では Child-Pugh A の患者のなかにも，門脈圧亢進症の患者が相当数含まれていることを報告した[11]。つまり，Child-Pugh A であるにもかかわらず，内視鏡所見にて red-color sign 陽性の食道静脈瘤や脾腫が発見された患者が相当数存在し，しかも若年であり通常の HCV 肝硬変とは異なる病態が存在することが示唆された(図 1)。

次に国内での HCV 単独感染者との予後の差異が報告されていなかったため，エイズ診療拠点病院である国立国際医療研究センター病院エイズ治療・研究開発センター(ACC)，国立病院機構大阪医療センター，横浜市立市民病院，国立病院機構九州医療センターの 4 施設にて，各施設での倫理委員会の承諾を得て，血友病に対して過去に使用された汚染血液製剤にて HIV/

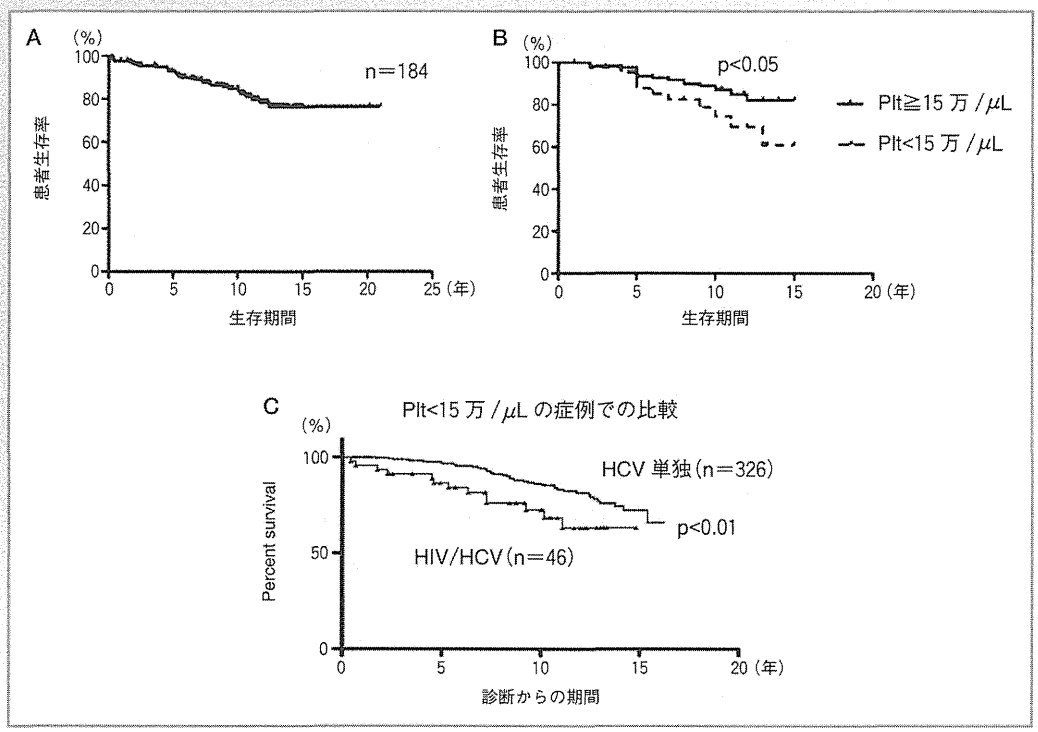


図 2　Child-Pugh A の HIV/HCV 重複感染者の患者生存率

(文献 28 より引用)

HCV 重複感染した患者の現在の血液生化学データ，および死亡者については死亡日を調査した[12]。エイズでの死亡を可及的除外するため ART が確立した 1997 年以降に HIV/HCV 重複感染と診断された患者(n＝184)を対象とした。血友病患者という特性上，プロトロンビン時間は測定されていないことが多く，この研究では Child-Pugh 分類ではなくプロトロンビン時間を項目に含まないオリジナルの Child 分類を採用した。最終的に計 184 例に関するデータを収集し，門脈圧亢進症の程度を簡便に反映できる血小板数が確認できた 171 例を 15 万/μL にて群別し，生存率を比較した。

その結果，血液製剤による HIV/HCV 重複感染者(n＝184)で Child 分類 A 患者生存率は 10 年で約 85 %であった(図 2-A，5 年 91.7 %，10 年 84.7 %，15 年 76.3 %)。しかし血小板数が確認できた 171 例を 15 万/μL 以上(n＝46)と未満(n＝125)で分別したところ，血液製剤による HIV/HCV 重複感染者の生存率は血小板数 15 万/μL 未満の症例で有意に不良であった(図 2-B)。さらに，この HIV/HCV 重複感染/Child 分類 A/ 血小板数 15 万/μL 未満の症例(n＝46)と，国立病院機構長崎医療センターにおける HCV 単独感染患者 Child 分類 A の症例(n＝326)との間で予後を比較したところ，HIV/HCV 重複感染患者の予後は有意に不良であった(図 2-C)。(HCV 単独：5 年生存率 96.7 %，10 年 86.2 %，15 年 72.4 %，HIV/HCV 重複：5 年生存率 87.7 %，10 年 74.5 %，15 年 60.1 %)。つまり HIV/HCV 重複感染では HCV 単独感染に比して，若年者であるのにもかかわらず，死亡率が上昇していることが明らかになった。肝移植適応時期に関しても HIV/HCV 重複感染の患者は HCV 単独感染の患者に比し，早期の肝移植が必要となる可能性が示唆された。また文献的にも Child-Pugh B, C の重複感染患者も同様に HCV 単独感染患者より予後不良であることが報告されている[13]。

## HIV/HCV 重複感染者に対する肝移植

### 1．肝移植の適応

HIV/HCV 重複感染者に対する肝移植の適応は，大

| Months | Survival percentage (group data) (95 %CI) | Sample size | Survival percentage (case data) (95 %CI) | Sample size |
|---|---|---|---|---|
| (a) Patient survival percentages | | | | |
| 12 | 84.49 (81.11–87.86) | 442 | 83.33 (78.19–88.47) | 149 |
| 24 | 73.52 (69.80–77.24) | 541 | 73.76 (67.17–80.35) | 89 |
| 36 | 66.17 (61.67–70.66) | 426 | 68.74 (61.25–76.23) | 54 |
| 48 | 66.69 (61.17–72.21) | 280 | 62.65 (53.16–72.14) | 26 |
| 60 | 63.75 (57.12–70.38) | 202 | 55.93 (43.56–68.30) | 13 |

肝移植後　1年生存率 84.5 %　5年生存率 63.8 %

図3　HIV陽性患者に対する肝移植成績(メタ解析)

(文献29より引用)

きく2つの病態，①非代償性C型肝硬変，②NCPH，に分けられると考えられる。

HIV陽性患者に対する肝移植の適応に関しては，欧米では，最新のUS National Institutes of Health (NIH)で以下のように定めている[14]。①一般的な肝移植の適応を満たしている，②CD4陽性T細胞数＞100個/μL(日和見感染の既往がある場合は＞200個/μL)，③HIV-RNA検出感度以下(viral load ＜ 50 copies/mL，超高感度amplicor monitor PCR使用)，④エイズを発症していない。特に進行性多巣性白質脳症，慢性クリプトスポリジウム腸炎(1ヵ月以上)，原発性中枢神経リンパ腫の既往がない。もちろん，活動性の感染症があれば絶対禁忌であるが，日和見感染症の既往に関しては，術後に対策可能な状況であれば適応としてよいとされている。禁忌となるのは，ARTで制御できない多剤耐性HIV，慢性クリプトスポリジウム腸炎，進行性多巣性白質脳症，そしてリンパ腫である。以前は，CD4陽性T細胞数250個/μL以上とする，という厳しい条件が求められていたが，門脈圧亢進症による脾機能亢進症が存在する病態下では100個/μL以上としても許容される，ということが現在では一般化されている[15]。また，特に肝硬変の症例では脾機能亢進に伴いT細胞の絶対数は減少するため，CD4/CD8比も参考にする。CD4/CD8 ＜ 0.15で腹部手術後合併症や日和見感染の発症が上昇するとするデータがあり[16][17]，これを基準としたガイドラインを専攻の兼松班にて上梓している。

HIV陽性患者に対する肝移植成績としては，通常のHIVなし，HCVなしの肝移植患者の5年生存率を約70 %，HCV感染者に対する肝移植での5年生存率を約60 %とすると，HIV/HCV重複感染患者では5年生存率約50 %と約10 %低下することが報告されている(図3)[18][19]。この5年生存率は国際的にも許容されており，また特に血液製剤によるHIV/HCV重複感染患者では救済医療の側面もあるため，本邦でも十分治療手段の1つとして考えられる。本邦でもすでに生体肝移植が10例程に施行されているが，元来血友病の家系であり，親族にも適格ドナーが存在しないことも多い。疫学的には，血液製剤によるHIV/HCV重複感染患者ですでに肝硬変となっている患者約50例，また慢性肝炎患者400例とされているが，概算では今のところ年間2-3名の患者が肝移植の適応になる可能性がある[20]。Child分類Aでも前述した早期増悪可能性を考慮し，希望があれば肝移植待機患者登録を考慮してもよいと考えられた。

通常のHCVによる肝硬変患者の肝移植後の免疫抑制はカルシニューリン阻害薬(CNI：タクロリムス水和物，シクロスポリン)，ステロイドで導入し，その後ステロイドを漸減し，CNI単剤で維持することが多い。またはステロイドを使用せず，抗CD25抗体を併用し，CNIのみで導入するレジメンもある。一方，HIV/HCV重複感染者の場合は多剤併用療法(HAART)薬剤とCNIの代謝酵素が拮抗するため，CNIの血中濃度が異常高値となることが多い。海外の報告によると，通常のタクロリムス水和物の投与期間，投与量を調節することで，HAART薬剤との併用

療法が行われることが多かったようである。本研究班と共同研究を行ったマイアミ大学での実際の個々の患者の免疫抑制剤の投与量，トラフ値とHAART薬剤の投与タイミングをみると，各症例でトラフ値はさまざまであったが，前期は過剰投与によるタクロリムス水和物トラフ値のovershoot傾向がみられた[21]。後期は，おそらく経験値の上昇により，1週間に1回投与などの工夫により通常の免疫抑制レベルで落ち着いている症例が多かった。最近CNIと代謝拮抗しない新しいHAART薬剤が開発され，その移植患者における使用が報告され始めてきている。ラルテグラビルカリウム(RAL)は新規インテグラーゼ阻害薬であり，CYP450と関与しない。RALを用いた免疫抑制療法が8人の肝移植，5人の腎移植患者に行われた報告では，CNIのトラフはHIV陰性の患者と同様な設定で行われ，RALのtolerabilityは良好であったとされている[22]。9ヵ月の観察期間で全例グラフト状態良好で，RAL+2核酸系逆転写酵素阻害薬(NRTI)によるHAART療法の有効性が示唆されている。

また，肝毒性も少ないEnfuvirtideも同様に使用されている。11例の肝移植例で使用され，対照群と比し，術後CD値，移植後肝機能データも遜色なく，CNIのコントロールが良好であったとされている[23]。ほかにはシロリムス単剤への変更による免疫抑制[24]やステロイド不使用による移植後感染症率低下を示す報告もある[25]。このように種々の薬剤，プロトコルの開発にて今後の移植成績の向上が望まれる。

## 最新のわれわれの研究

2009年より厚生労働科学研究費補助金エイズ対策研究事業「血液製剤によるHIV/HCV重複感染患者に対する肝移植のための組織構築」(兼松班)，2012年より「血液製剤によるHIV/HCV重複感染者に対する肝移植適応に関する研究」(指定研究　江口班)にて研究を行ってきた。

兼松班では，前述のように血液製剤によるHIV/HCV重複感染患者においては，みかけの肝機能は良好であるが門脈圧亢進症の所見が強く，HCV単独感染とは異なる病態であることを明らかとした。つまり，HIV/HCV重複感染者ではChild-Pugh Aにもかかわらず門脈圧亢進症による脾腫(汎血球減少)，胃食道静脈瘤，肝性脳症が前面に現れる病態が存在し，NCPHと呼ばれる病態である[2]。当該患者の最近の肝機能，肝予備能，門脈圧亢進症状を検討したところ，肝機能自体はChild-Pugh Aであるのにもかかわらず，門脈圧亢進の所見を呈する症例が約60％に存在した[3]。このことは健康危険情報としてAPI NET (AIDS Prevention Information Network)でも報告した[3]。原因としては，比較対照研究にてARTによるもの，特にdidanosineが推測されている[9]。薬剤性の，いわゆるveno-occlusive disease (VOD)やsinusoidal obstruction syndrome (SOS)と言われる病態と類似しているものと思われるが[4) 5]，HCVによる肝障害にこれらの病態が加わることにより，一度でも消化管出血や脳症などのエピソードが出現するときわめて予後不良となることも報告されており[10) 11]，通常のHCV肝硬変よりも肝移殖の適応を早期に考慮する必要がある。Child-Pugh Aの患者であっても，HIV/HCV重複感染者で門脈圧亢進症が進行する例では，自然予後は不良であり，感染時期(生下，幼少時)からの年数を考慮すると若年での死亡も考えられた。その結果，ガイドラインを上梓し(図4)，Child-Pugh Aの患者さんにおいても門脈圧亢進症状が強い例では，肝移植施設へのコンサルトが必要であることを推奨した。血友病，HIV感染，HCV感染など多角的な肝移植周術期管理の必要性とその詳細を記載している[26]。

その後の江口班では，さまざまなエビデンスを集め，HCV単独感染例との比較によりHIV/HCV重複感染者の予後が不良，肝線維化の進展が早いことを示した。非侵襲的な検査であるARFI (acoustic radiation force impulse imaging)による肝硬度やAPRI (AST-platelet ratio index)；(AST/AST正常上限［IU/L］)／血小板数［$\times 10^9$/L］$\times$ 100)と患者予後との関連を検討したところ，重複感染患者はHCV単独感染患者よりも3倍速く線維化が進行する可能性が示唆された[27]。これらの結果をもとに，通常緊急度で3点(Child-Pugh B)・6点・8点(Child-Pugh C)・10点(劇症肝不全などの超緊急症例)とされているポイントを，HIV陽性

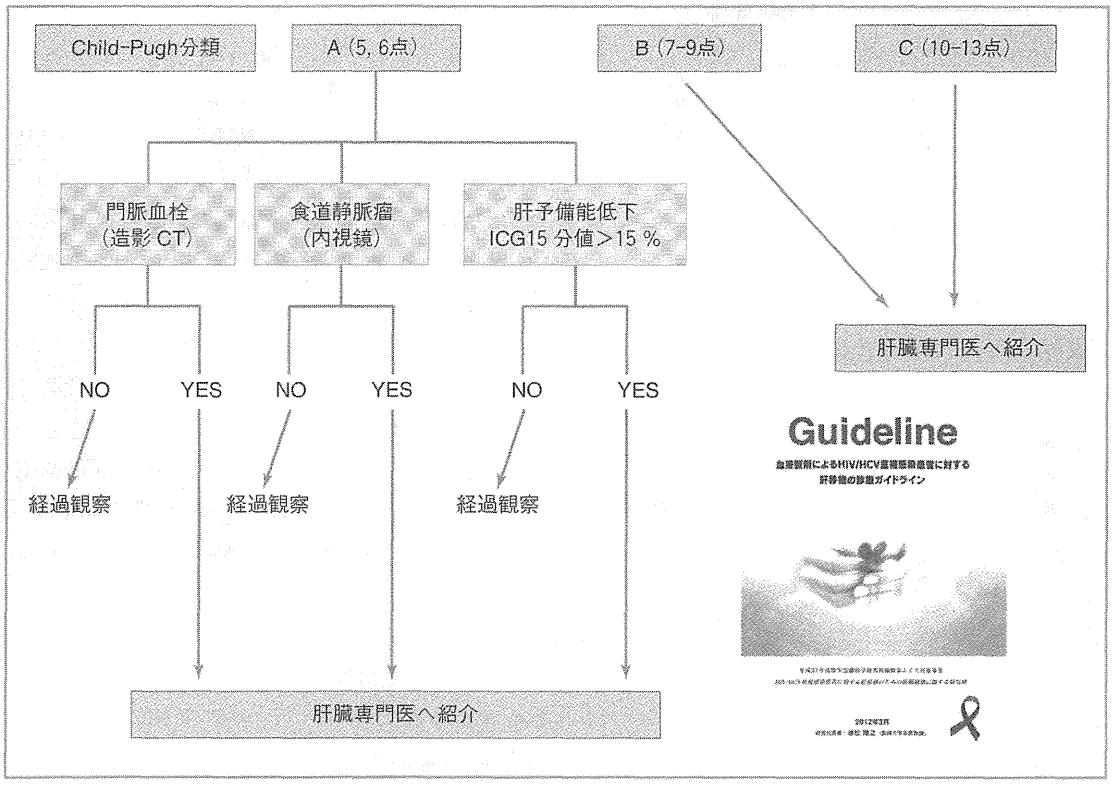


図 4　HIV/HCV 重複感染患者における肝疾患サーベイランスアルゴリズム
（文献 26 より作図）

資料 1　医学的緊急度のランクアップ

1．HIV と HCV 共感染者における死亡原因の大多数が肝不全死であり，Child A, Child B の病態で肝不全，食道静脈瘤破裂などによる割合が感染症より増加している。そのため，長崎大学の兼松班，江口班のまとめにより，以下のように医学的緊急度をランクアップした。

HIV/HCV 共感染者の Child A は Child B 相当として緊急度 3 点，Child B は Child C 相当として緊急度 6 点，Child C は通常緊急度 6 点であるが，この場合 Child スコア 13 点以上，MELD25 点以上の緊急度 8 点相当とする。

2．小児の原発性硬化性胆管炎に対する生体肝移植の症例は予後が悪く，原発性硬化性胆管炎全体でも脳死肝移植に比して生体肝移植の成績が悪いことが判明している。

そこで，特に小児の原発性硬化性胆管炎に関してはボーナスポイントとして下記のように緊急度をランクアップした。

小児の原発性硬化性胆管炎の Child A は Child B 相当として緊急度 3 点，Child B は Child C 相当として緊急度 6 点，Child C は通常緊急度 6 点ですが，この場合 Child スコア 13 点以上，MELD25 点以上の緊急度 8 点相当とする。

図 5　HIV/HCV 重複感染患者に対する脳死肝移植登録について
（日本脳死肝移植適応評価委員会 2012 年 9 月）

患者で条件を満たせばランクアップし，Child-Pugh Aでも門脈圧亢進症の所見があれば登録できるようにすべき，として3点(Child-Pugh A)，6点・8点(Child-Pugh B/C)で登録することを提言し，脳死肝移植適応評価委員会，肝移植研究会と議論のうえ，承認された(図5)。この新基準が2014年2月に全国の肝移植施設へ通知され，現在3例が登録待機中である。

## おわりに

HIV/HCV重複感染患者はHCV単独感染患者と同様の適応では救命は難しいと考えられ，門脈圧亢進症がみられればChild-Pugh Aでもその時点で肝移植を考慮すべきである。また，Child-Pugh B，Cの患者においても，HCV単独感染患者より予後不良であることから，患者の希望があれば早期肝移植専門施設へのコンサルトが重要であると考えられた。

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***Original Article***

# Impact of branched-chain amino acid supplementation on survival in patients with advanced hepatocellular carcinoma treated with sorafenib: A multicenter retrospective cohort study

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*Aim:* The therapeutic efficacy of branched-chain amino acid (BCAA) when added to sorafenib has not been fully assessed in patients with advanced hepatocellular carcinoma (HCC). This multicenter study investigated whether BCAA supplementation improves prognosis in patients with advanced HCC who underwent sorafenib treatment.

*Methods:* This retrospective analysis included 256 patients with advanced HCC treated with sorafenib, including 55 who did and 201 who did not receive BCAA supplementation. Clinical characteristics and outcomes in relation to Child–Pugh classification were compared in the two groups. Statistical analyses of univariate, multivariate and propensity score-based procedures were used for this study.

*Results:* Assessment of 216 Child–Pugh A patients showed that median overall survival was significantly longer in patients with BCAA supplementation than in those without it (440 vs 299 days, $P = 0.023$). Multivariate analysis showed that BCAA supplementation ($P = 0.023$), low α-fetoprotein (<100 ng/mL) ($P < 0.001$), less progressive Barcelona Clinic Liver Cancer stage (A and B) ($P = 0.007$) and male sex ($P = 0.018$) were significant independent contributors to better overall survival. The significantly longer overall survival by BCAA supplementation was verified in the analysis using the propensity score in combination with the inverse probability of treatment weighted adjustment ($P = 0.026$). Assessment of the 40 Child–Pugh B patients showed no significant differences in overall survival between patients with and without BCAA supplementation.

*Conclusion:* BCAA supplementation may be a valuable adjunctive therapy for improving prognosis in sorafenib-treated Child–Pugh A patients with advanced HCC.

**Key words:** advanced hepatocellular carcinoma, branched-chain amino acid supplementation, overall survival, propensity score-based statistical analysis, sorafenib

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## INTRODUCTION

LIVER CANCER IS the fifth most common cancer in males and the seventh most common in females worldwide, with most of these patients having hepatocellular carcinoma (HCC).[1] Treatments for HCC include surgical resection, locoregional ablation and transarterial embolization, but these therapeutic modalities are not generally indicated for patients with advanced HCC, resulting in poor patient prognosis. Two phase 3 randomized controlled trials revealed that sorafenib, an oral multikinase inhibitor with antiproliferative and antiangiogenic activities, prolonged the time to progression and overall survival in patients with advanced HCC.[2,3] Sorafenib is currently recognized as a standard therapeutic regimen for advanced HCC.

A reduction in the serum concentrations of branched-chain amino acids (BCAA), namely, valine, leucine and isoleucine, is one of the distinctive clinical features of chronic liver diseases, especially cirrhosis, which is involved in the pathogenesis of protein–energy malnutrition and hepatic encephalopathy.[4] Recently, BCAA has drawn attention as a pharmacological nutritive factor in patients with chronic liver diseases.[4] Two randomized controlled trials from Italy and Japan reported that supplementation with BCAA reduced the duration of cirrhosis-related hospitalization, as well as cirrhosis-related deaths.[5,6] Subgroup analyses of patients in the Japanese trial revealed that BCAA may also reduce hepatocarcinogenesis, although this effect was limited to cirrhotic patients with obesity and those with chronic hepatitis C virus infection.[7] A more recent prospective study reported that administration of BCAA may reduce the risk of HCC in cirrhotic patients.[8] BCAA has also been shown to prevent HCC recurrence after surgical resection and radiofrequency ablation.[9,10] Taken together, these findings indicate that BCAA supplementation may prevent the development or recurrence of HCC and improve prognosis in patients with chronic liver disease. A single-center study involving a relatively small number of patients found that the addition of BCAA to sorafenib prolonged the duration of sorafenib therapy and overall survival in patients with HCC.[11] However, further investigations, in larger numbers of patients, are required to assess the therapeutic efficacy of adding BCAA to sorafenib in patients with advanced HCC. This multicenter retrospective cohort study therefore investigated whether BCAA supplementation would improve prognosis in sorafenib-treated patients with advanced HCC.

## METHODS

### Patients

THIS STUDY INVOLVED 273 consecutive patients with advanced HCC treated with sorafenib between January 2007 and December 2012 at 14 institutions participating in the Osaka Liver Forum and located around the Osaka distinct in Japan. Of these, 17 patients were administrated BCAA granules (Livact; Ajinomoto Pharmaceuticals, Tokyo, Japan) for only part of the duration of treatment with sorafenib, with most administrated BCAA owing to a reduction in liver functional reserve after the start of sorafenib treatment; these 17 patients were excluded. Thus, 256 patients were included in this study, including 55 patients who were and 201 who were not treated with BCAA. Patients in the BCAA group were administrated BCAA granules (Livact) throughout the entire period of sorafenib administration, whereas patients in the non-BCAA group did not receive BCAA granules during sorafenib treatment.

The study protocol was in accordance with the Declaration of Helsinki and was approved by the institutional review board at each participating institution.

### Diagnosis of HCC

All 256 patients had advanced HCC, with none deemed eligible for surgical resection, locoregional ablation or transarterial embolization. In most patients, HCC was diagnosed by dynamic contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) based on enhancement in the arterial phase and low density/intensity in the portal/equilibrium and hepatobiliary phases. If necessary, patients were diagnosed by other imaging modalities, including CT angiography and contrast-enhanced ultrasound or by histological examination of tumor biopsy specimens.

### Drugs

Sorafenib (Nexavar; Bayer Pharmaceuticals, Osaka, Japan) was administrated at an initial dose of 200 mg/day to eight patients (3%), at 400 mg/day to 124 (48%) patients, at 600 mg/day to 14 patients (5%) and at 800 mg/day to 110 patients (43%). Dose reduction was based on the degree of drug toxicity. Sachets of BCAA granules (Livact), each containing 952 mg L-isoleucine, 1904 mg L-leucine and 1144 mg L-valine, were administrated three times per day.

## Follow up

Patients were followed up at intervals of 2–4 weeks by physical examinations and blood tests. CT or MRI was performed every 4–8 weeks, and the treatment response of sorafenib was assessed according to modified Response Evaluation Criteria in Solid Tumors (mRECIST) version 1.1.[13,14] Treatment was terminated owing to disease progression or intolerable sorafenib-related adverse events.

## Statistical analyses

Data were analyzed using the IBM SPSS program version 21.0 and R for Windows 3.2.0 (SPSS, Chicago, IL, USA). The two groups were compared by Fisher's exact tests, $\chi^2$-tests, Student's *t*-tests and Mann–Whitney non-parametric *U*-tests, as appropriate, with *P*-values less than 0.05 considered significant. Cumulative overall survival in the two groups was determined using the Kaplan–Meier method and compared using the log–rank test. The contribution of various clinical factors to overall survival was evaluated by a Cox proportional hazards model using univariate and multivariate procedures. Only variables with *P*-values less than 0.05 in univariate analysis were included in multivariate analysis.

The propensity score for BCAA supplementation were calculated by multivariate logistic regression analysis, adjusted for 11 covariates, namely, age, sex, hepatitis B positivity, hepatitis C positivity, Barcelona Clinic Liver Cancer (BCLC) stage, platelet count, albumin, alanine aminotransferase, prothrombin time, α-fetoprotein (AFP; <100 vs ≥100 ng/mL) and des-γ-carboxyprothrombin (DCP; <400 vs ≥400 mAU/mL). For the inverse probability of treatment weighted adjustment, weights for patients in the BCAA group were the inverse of propensity scores, and weights for patients in the non-BCAA group were the inverse of (1-propensity scores). Because a wide range of weights may have caused the inaccurate or unstable result, the trimming method excluding the lower and the upper bands of 3 standard deviations (SD) from the mean weight was used in this analysis. These values were then incorporated into the weighted Cox regression model to evaluate overall survival.

# RESULTS

## Clinical features and outcome of sorafenib-treated HCC patients

THE 256 PATIENTS included 212 men and 44 women, with a mean age of 72.0 ± 9.0 years. Underlying liver diseases included hepatitis C (as determined by positivity for antibody to hepatitis C virus) in 141 (55%) patients, hepatitis B (as determined by positivity for hepatitis B virus surface antigen) in 50 patients (20%), and both in three patients (1%). The remaining 62 (24%) had neither hepatitis B nor C, 30 of whom had alcoholic liver disease. As for antiviral treatment for hepatitis viruses, 25 (50%) of the 50 patients with hepatitis B virus infection had received the continuous treatment with the nucleoside analog entecavir. Interferon-based antiviral treatment had been carried out in 29 (21%) of the 141 patients with hepatitis C virus infection, nine of whom had achieved the sustained eradication of the virus. Diabetes mellitus, as judged by the fasting blood glucose of 126 mg/dL or more or administration of diabetes drug, was found in 77 patients (30%). BCLC staging[12] of HCC lesions was A in nine patients (4%), B in 65 (25%) and C in 182 (71%). Portal invasion was observed in 79 patients (31%) and extrahepatic metastasis in 90 (35%). Of the 256 patients, 216 (84%) were classified as Child–Pugh A and 40 (16%) as Child–Pugh B. Two hundred and thirty-nine patients (93%) had been previously treated for HCC, hepatic resection in 66 (26%), radiofrequency ablation in 116 (45%), percutaneous ethanol injection in 51 (20%) and transcatheter arterial embolization in 218 (85%). Eighty (31%) patients had been treated with other therapies, including microwave coagulation, radiation and hepatic arterial infusion chemotherapy. For life-threatening complications, concomitant malignancies of other organs were seen in 13 (5%) patients. There were 36 patients (14%) who had serious infectious diseases during follow up. The mean duration of sorafenib therapy was 132 ± 151 days, and the mean follow-up period was 259 ± 214 days.

In comparing the clinical features of the 216 Child–Pugh A patients who were and were not treated with BCAA (Table 1), we found that platelet count (10.9 ± 5.5 vs 14.9 ± 6.7 × $10^4$/$mm^3$, $P = 0.001$), albumin (3.4 ± 0.4 vs 3.7 ± 0.4 g/dL, $P < 0.001$) and prothrombin time (79 ± 11% vs 88 ± 11%, $P < 0.001$) were significantly lower in the 37 patients in the BCAA group than in the 179 in the non-BCAA group. Thus, in Child–Pugh A patients, BCAA group patients tended to have lower liver functional reserve than non-BCAA group ones. In contrast, age, sex ratio, etiology of underlying liver disease, body mass index, diabetes mellitus, BCLC stage, portal vein invasion, extrahepatic metastasis, alanine aminotransferase, AFP, DCP, types of previous treatment for HCC and initial dose of sorafenib did not differ significantly in the BCAA and non-BCAA groups. There were no significant differences in the frequencies of antiviral treatment for hepatitis B and C viruses, concomitant

**Table 1** Clinical features of HCC patients treated with sorafenib belonging to BCAA and non-BCAA groups in relation to Child-Pugh classification

| Clinical features | Child–Pugh A | | | | Child–Pugh B | | | |
|---|---|---|---|---|---|---|---|---|
| | Total | BCAA | Non-BCAA | *P* | Total | BCAA | Non-BCAA | *P* |
| *n* | 216 | 37 | 179 | – | 40 | 18 | 22 | – |
| Age (years) | 72.4 ± 8.6[†] | 72.2 ± 7.8 | 72.4 ± 8.8 | NS | 69.8 ± 10.9 | 73.1 ± 6.4 | 67.2 ± 13.0 | NS |
| Sex (male/female) | 180/36 | 31/6 | 149/30 | NS | 32/8 | 14/4 | 18/4 | NS |
| Etiology (hepatitis B/hepatitis C/hepatitis B and C/non-B, non-C | 41/120/3/52 | 4/26/0/7 | 37/94/3/45 | NS | 9/21/0/10 | 3/10/0/5 | 6/11/0/5 | NS |
| Body mass index (kg/m$^2$) | 22.8 ± 3.4 | 22.2 ± 3.0 | 22.9 ± 3.5 | NS | 23.7 ± 3.4 | 24.6 ± 3.4 | 23.0 ± 3.2 | NS |
| Diabetes mellitus (absent/present) | 153/63 | 29/8 | 124/55 | NS | 26/14 | 11/7 | 15/7 | NS |
| BCLC stage (A/B/C) | 9/55/152 | 0/9/28 | 9/46/124 | NS | 0/10/30 | 0/4/14 | 0/6/16 | NS |
| Portal vein invasion (absent/present) | 149/67 | 23/14 | 126/53 | NS | 28/12 | 14/4 | 14/8 | NS |
| Extrahepatic metastasis (absent/present) | 138/78 | 21/16 | 117.62 | NS | 28/12 | 13/5 | 15/7 | NS |
| Platelet count (×10$^4$/mm$^3$ | 14.2 ± 6.8 | 10.9 ± 5.5 | 14.9 ± 6.7 | 0.001 | 10.3 ± 4.0 | 10.8 ± 3.5 | 10.0 ± 4.4 | NS |
| Albumin (g/dL) | 3.6 ± 0.4 | 3.4 ± 0.4 | 3.7 ± 0.4 | <0.001 | 3.1 ± 0.4 | 3.1 ± 0.4 | 3.1 ± 0.4 | NS |
| Alanine aminotransferase (U/L) | 41 ± 28 | 42 ± 20 | 42 ± 37 | NS | 43 ± 24 | 42 ± 26 | 44 ± 22 | NS |
| Total bilirubin (mg/dL) | 0.8 ± 0.4 | 1.0 ± 0.4 | 0.8 ± 0.3 | NS | 1.3 ± 0.6 | 1.2 ± 0.5 | 1.4 ± 0.8 | NS |
| Prothrombin time (%) | 86 ± 12 | 79 ± 11 | 88 ± 11 | <0.001 | 75 ± 11 | 77 ± 13 | 72 ± 8 | NS |
| AFP (ng/mL) | 136 (≤5–≥100 000)[‡] | 128 (≤5–41 148) | 167 (≤5–≥100 000) | NS | 424 (≤5–≥100 000) | 354 (≤5–9962) | 828 (≤5–≥100 000) | NS |
| DCP (mAU/mL) | 610 (≤30–≥750 000) | 290 (≤30–≥750 000) | 664 (≤30–≥750 000) | NS | 507 (≤30–≥750 000) | 504 (≤30–≥750 000) | 781 (≤30–220 000) | NS |
| Previous therapy | | | | | | | | |
| Hepatic resection | 56 | 11 | 45 | NS | 10 | 3 | 7 | NS |
| Radiofrequency ablation | 96 | 17 | 79 | NS | 20 | 10 | 10 | NS |
| Percutaneous ethanol injection | 43 | 9 | 34 | NS | 8 | 5 | 3 | NS |
| Transcatheter arterial embolization | 181 | 33 | 148 | NS | 37 | 18 | 19 | NS |
| Others | 66 | 10 | 56 | NS | 14 | 5 | 9 | NS |
| Initial dose of sorafenib per day (200/400/600/800 mg) | 6/101/13/96 | 1/19/3/14 | 5/82/10/82 | NS | 2/23/1/14 | 0/10/1/7 | 2/13/0/7 | NS |
| Period of sorafenib therapy (days) | 136 ± 154 | 176 ± 180 | 128 ± 146 | NS[*] | 107 ± 135 | 86 ± 76 | 123 ± 168 | NS |

[†]Values are expressed as mean ± standard deviation.

[‡]Values are expressed as median (range).

[*]$P = 0.084$.

AFP, α-fetoprotein; BCAA, branched-chain amino acid; BCLC, Barcelona Clinic Liver Cancer; DCP, des-γ-carboxyprothrombin; SD, standard deviation.

malignancies of other organs and serious infectious diseases between the BCAA and non-BCAA groups (data not shown). The duration of sorafenib therapy tended to be longer in the BCAA than in the non-BCAA group (176 ± 180 vs 128 ± 146 days, $P = 0.084$), although the difference was not statistically significant.

Comparisons in the 40 Child–Pugh B patients who did ($n = 18$) and did not ($n = 22$) receive BCAA supplementation showed no significant difference in any demographic or clinical characteristic.

During follow up, there were 109 deaths in Child–Pugh A patients and 26 deaths in Child–Pugh B patients. In Child–Pugh A patients, 14 (93%) of 15 deaths in the BCAA group compared with 89 (95%) of 94 deaths in the non-BCAA group were related to liver disease (not significant). Also, in Child–Pugh B patients, no significant differences were observed in the rate of liver disease-related deaths between the BCAA group (10/11, 91%) and the non-BCAA group (15/15, 100%).

## Effects of BCAA supplementation on overall survival

Kaplan–Meier analysis of overall survival in sorafenib-treated patients with advanced HCC and Child–Pugh A status showed that median overall survival was significantly longer in the BCAA than in the non-BCAA group (440 vs 299 days, $P = 0.023$) (Fig. 1, left panel). In Child–Pugh B patients, however, median overall survival did not differ significantly in the BCAA and non-BCAA groups (256 vs 156 days) (Fig. 1, right panel).

Among the 216 Child–Pugh A patients, the antitumor effect, as determined by mRECIST version 1.1 criteria,[12,13] could be evaluated in 168 patients, 28 in the BCAA and 140 in the non-BCAA group. Of the 28 patients in the BCAA group, one (4%) achieved a partial response, 18 (64%) had stable disease and nine (32%) had progressive disease. In the non-BCAA group, 11 patients (8%) achieved a partial response, 64 (46%) had stable disease and 65 (46%) had progressive disease. The overall response rates (4% vs 8%) and disease control rates (68% vs 54%) did not differ significantly in the BCAA and non-BCAA groups.

Analysis of Child–Pugh A patients showed that sorafenib therapy was halted owing to adverse events in 17 of the 37 patients (46%) in the BCAA group and in 85 of the 179 (47%) in the non-BCAA group (not significant). The main adverse events resulting in therapy cessation were hand–foot syndrome (10 patients), rash/erythema multiforme (11 patients), liver dysfunction (20 patients), gastrointestinal bleeding (10 patients), general fatigue (eight patients), anorexia (six patients), ascites (five patients), infection (five patients), diarrhea (four patients) and renal dysfunction (three patients). The frequencies of these adverse events did not differ in the BCAA and non-BCAA groups (data not shown).

## Factors contributing to overall survival

Factors contributing to overall survival were analyzed using univariate and multivariate Cox proportional hazards models (Table 2). Factors analyzed included age,

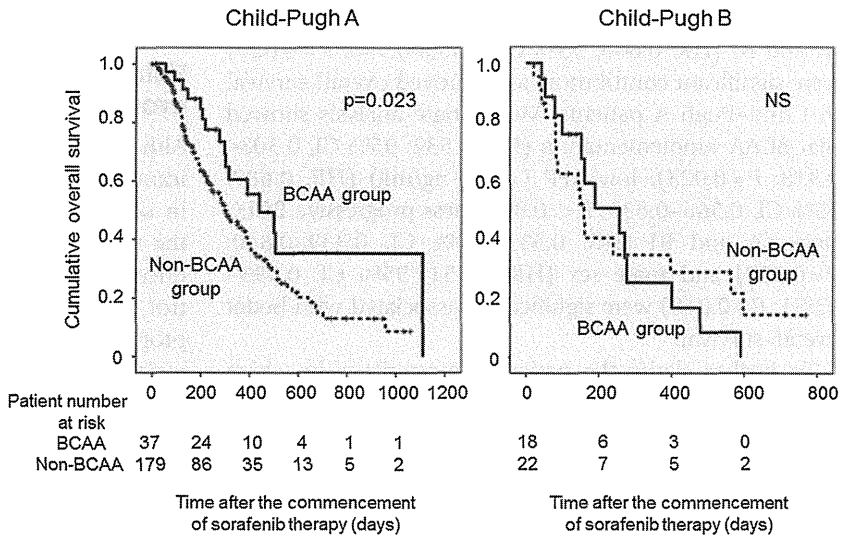


**Figure 1** Cumulative overall survival in sorafenib-treated hepatocellular carcinoma patients with and without branched-chain amino acid (BCAA) supplementation. Left panel, Child–Pugh A patients; right panel, Child–Pugh B patients. Solid lines, BCAA group; dotted lines, non-BCAA group.

**Table 2** Univariate and multivariate analyses to investigate factors contributing to overall survival in HCC patients treated with sorafenib of Child–Pugh A

| Variables | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *P* | HR | 95% CI | *P* |
| Age (/1-year increment) | 1.005 | 0.983–1.028 | 0.628 | – | – | – |
| Sex (male) | 0.548 | 0.346–0.868 | 0.010 | 0.751 | 0.593–0.951 | 0.018 |
| Hepatitis B (positive) | 1.125 | 0.735–1.723 | 0.588 | – | – | – |
| Hepatitis C (positive) | 1.107 | 0.766–1.599 | 0.588 | – | – | – |
| Body mass index (/1-kg/m$^2$ increment) | 0.992 | 0.940–1.047 | 0.771 | – | – | – |
| Diabetes (present) | 1.292 | 0.873–1.912 | 0.200 | – | – | – |
| BCLC stage (A and B) | 0.608 | 0.389–0.950 | 0.029 | 0.533 | 0.339–0.840 | 0.007 |
| Portal vein invasion (present) | 1.318 | 0.896–1.940 | 0.161 | – | – | – |
| Extrahepatic metastasis (present) | 1.260 | 0.862–1.843 | 0.233 | – | – | – |
| Platelet count (/1 × 10$^4$/mm$^3$ increment) | 1.027 | 1.000–1.056 | 0.051 | – | – | – |
| Albumin (/1-g/dL increment) | 0.920 | 0.599–1.412 | 0.702 | – | – | – |
| Alanine aminotransferase (1-U/L increment) | 1.002 | 0.996–1.009 | 0.436 | – | – | – |
| Total bilirubin (/1-mg/dL increment) | 0.937 | 0.580–1.513 | 0.789 | – | – | – |
| Prothrombin time | 1.007 | 0.992–1.024 | 0.353 | – | – | – |
| AFP (<100 ng/mL) | 0.501 | 0.340–0.739 | <0.001 | 0.689 | 0.566–0.839 | <0.001 |
| DCP (<400 mAu/mL) | 0.724 | 0.492–1.065 | 0.101 | – | – | – |
| BCAA supplement (yes) | 0.538 | 0.313–0.925 | 0.025 | 0.532 | 0.308–0.918 | 0.023 |

AFP, α-fetoprotein; BCAA, branched-chain amino acid; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; DCP, des-γ-carboxyprothrombin; HCC, hepatocellular carcinoma; HR, hazard ratio; SD, standard deviation.

sex, hepatitis B and hepatitis C positivity, body mass index, diabetes mellitus, BCLC stage (A and B vs C), portal vein invasion, extrahepatic metastasis, platelet count, albumin, alanine aminotransferase, total bilirubin, prothrombin time, AFP (<100 vs ≥100 ng/mL), DCP (<400 vs ≥400 mAU/mL) and BCAA supplementation. Univariate analysis showed that BCAA supplementation (hazard ratio [HR], 0.538; 95% confidence interval [CI], 0.313–0.925; $P=0.025$), low AFP (<100 ng/mL) (HR, 0.501; 95% CI, 0.340–0.739; $P< 0.001$), male sex (HR, 0.548; 95% CI, 0.346–0.868; $P=0.010$) and less progressive BCLC stage (A and B) (HR, 0.608; 95% CI, 0.389–0.950; $P=0.029$) were significant contributors to improved overall survival in Child–Pugh A patients. Multivariate analysis showed that BCAA supplementation (HR, 0.532; 95% CI, 0.308–0.918; $P=0.023$), low AFP (<100 ng/mL) (HR, 0.689; 95% CI, 0.566–0.839; $P< 0.001$), less progressive BCLC stage (A and B) (HR, 0.533; 95% CI, 0.339–0.840; $P=0.007$) and male sex (HR, 0.751; 95% CI, 0.593–0.951; $P=0.018$) were significantly associated with better overall survival.

To further clarify the patient characteristics in which BCAA supplementation was more effective, univariate Cox regression analysis to investigate the contribution of BCAA supplementation to overall survival was carried out stratified by sex, BCLC stage and AFP in HCC patients of Child–Pugh A (Table 3). The effect of BCAA supplementation on overall survival was evident in patients with AFP of 100 ng/mL or more (HR, 0.504; 95% CI, 0.258–0.984; $P=0.045$). The tendency of survival advantage by BCAA supplementation was also seen in male patients (HR, 0.602; 95% CI, 0.335–1.081; $P=0.089$) and patients of BCLC stage A and B (HR, 0.284; 95% CI, 0.067–1.211; $P=0.089$), although the differences did not reach a statistically significant level.

Analysis of the Child–Pugh B patients showed that no factors significantly influenced overall survival (data not shown).

## Validation of survival advantage by means of propensity score-based statistical methods

Although BCAA supplementation was found to be a significant independent predictor of prolonged overall survival in sorafenib-treated Child–Pugh A patients with HCC, the retrospective design of this study might have introduced selection bias due to confounding factors. To control for these confounders, statistical analysis using the propensity score and the inverse probability of treatment weighted adjustment was employed to assess the survival benefits of BCAA supplementation. Propensity scores were calculated in each Child–Pugh A patient based on 11 covariates; age, sex, hepatitis B and hepatitis C positivity, BCLC stage, platelet count, albumin, alanine aminotransferase, prothrombin time, AFP and DCP. In the inverse

**Table 3** Univariate Cox regression analysis to investigate the contribution of BCAA supplementation to overall survival stratified by sex, BCLC stage and AFP in HCC patients treated with sorafenib of Child–Pugh A

| Variables of stratification/ subgroups | HR | 95% CI | *P* |
|---|---|---|---|
| All patients | 0.538 | 0.313–0.925 | 0.025 |
| Sex | | | |
| Male | 0.602 | 0.335–1.081 | 0.089 |
| Female | 0.311 | 0.072–1.345 | 0.118 |
| BCLC stage | | | |
| A and B | 0.284 | 0.067–1.211 | 0.089 |
| C | 0.701 | 0.388–1.268 | 0.240 |
| AFP | | | |
| <100 ng/mL | 0.659 | 0.257–1.691 | 0.386 |
| ≥100 ng/mL | 0.504 | 0.258–0.984 | 0.045 |

AFP, α-fetoprotein; BCAA, branched-chain amino acid; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; HCC, hepatocellular carcinoma; HR, hazard ratio.

probability of treatment weighted adjustment, one patient, whose weight was beyond the mean ± 3 SD, was regarded to have an outlier and trimmed for the analysis. The significantly longer overall survival was still observed in the BCAA than in the non-BCAA group (HR, 0.498; 95% CI, 0.269–0.922; $P=0.026$) in this propensity score-based analysis.

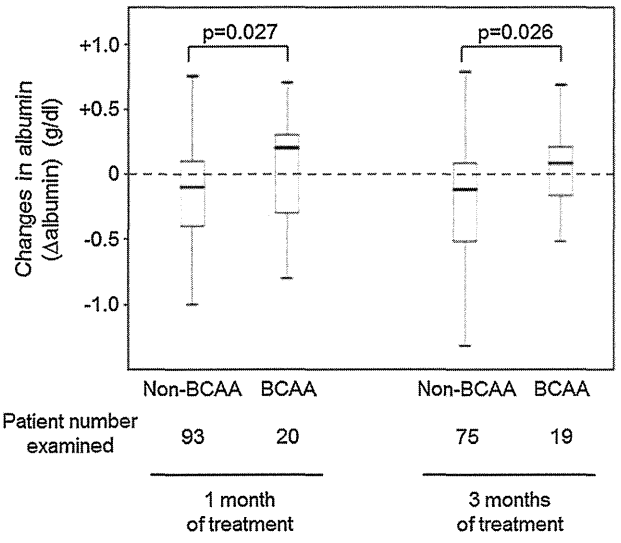


**Figure 2** Changes in the albumin level in sorafenib-treated hepatocellular carcinoma patients of Child–Pugh A with and without branched-chain amino acid (BCAA) supplementation. Changes in albumin were investigated at 1 and 3 months of sorafenib treatment. Δalbumin represents the albumin level after the commencement of sorafenib treatment minus that at baseline.

## Improvement of liver functional reserve by BCAA supplementation during sorafenib treatment

Finally, the changes in the liver functional reserve during sorafenib treatment were compared between HCC patients of Child–Pugh A with and without BCAA supplementation. Albumin was used as a surrogate for the liver functional reserve, and the analysis was carried out in a portion of patients whose albumin data after the commencement of sorafenib treatment were available. Changes in the albumin were represented by the Δalbumin (the albumin level after the commencement of sorafenib treatment minus that at baseline). As shown in Figure 2, the Δalbumin was higher in the BCAA group than in the non-BCAA group at 1 month ($0.1 \pm 0.4$ vs $-0.2. \pm 0.4$ g/dL, $P=0.027$) and 3 months ($0.1 \pm 0.4$ vs $-0.2. \pm 0.5$ g/dL, $P=0.026$) of treatment, suggesting that BCAA supplementation may maintain the liver functional reserve in sorafenib-treated HCC patients.

## DISCUSSION

SORAFENIB HAS BEEN approved worldwide as first-line therapy for patients with advanced HCC who are ineligible for surgical resection, locoregional ablation and transarterial embolization.[2,3] In particular, sorafenib is the only effective treatment currently available for HCC with extrahepatic metastasis. Supplementation with BCAA has been shown to reduce the risks of hepatocarcinogenesis and hepatic decompensation-related complications in patients with chronic liver diseases.[5–8] This multicenter retrospective study therefore investigated the effects of BCAA administration in patients with advanced HCC being treated with sorafenib.

In univariate and multivariate Cox proportional hazards analyses, BCAA supplementation, as well as low AFP, male sex and less progressive BCLC stage, were found to be significant independent factors associated with better overall survival in Child–Pugh A, but not Child–Pugh B, patients. The latter may be due to the poor prognosis of Child–Pugh B patients, masking any ameliorating effect of BCAA on survival.

The results of a retrospective observational study may be influenced by potential confounding founders and selection biases. This led us to perform statistical analysis using the propensity score in combination with the inverse probability of treatment weighted adjustment. As a result, we verified that BCAA supplementation continued to be associated with better prognosis in sorafenib-treated patients with HCC and Child–Pugh A status.

A comparison of baseline clinical characteristics in these Child–Pugh A patients with HCC showed lower liver functional reserve (lower prothrombin time and albumin) in the BCAA than in the non-BCAA group. Despite this difference, which indicated poorer prognosis in the BCAA group, patients in this group had significantly better overall survival. Taken together, these findings suggest that BCAA supplementation may have substantial clinical benefit for Child–Pugh A patients with advanced HCC being treated with sorafenib. A previous single-center study also reported that BCAA supplementation had survival advantages in sorafenib-treated patients with HCC.[11] Thus, our multicenter retrospective cohort study, which included a larger number of patients and utilized various statistical procedures to correct for confounding and selection biases, may strongly support this earlier finding.

The mechanism underlying the benefits of BCAA supplementation in these patients remains unclear. One of the major adverse events of sorafenib is the deterioration of liver function, which may result in treatment discontinuation. BCAA has been shown to remedy protein–energy malnutrition and increase liver functional reserve,[4] as well as to improve quality of life in patients with chronic liver diseases.[5] BCAA may also relieve generalized fatigue associated with sorafenib administration. Thus, BCAA supplementation may improve adherence to sorafenib by maintaining liver functional reserve and improving general health, resulting in longer term administration of sorafenib and the prolonged overall survival. Indeed, in our HCC patients of Child–Pugh A, patients receiving BCAA supplementation kept the albumin at a higher level during sorafenib treatment compared with those who did not receive it. Also, patients in our BCAA group tended to have a longer period of sorafenib administration than those in the non-BCAA group, although the difference was not statistically significant.

Branched-chain amino acid has been reported to modulate various cellular molecular pathways, including those involving mammalian target of rapamycin,[15–17] liver X receptor-α,[18] sterol regulatory element-binding protein-1c[18] and peroxisome proliferator-activated receptor-α,[19] resulting in enhanced production of albumin[15] and improvement of insulin resistance.[20,21] In addition, sorafenib and BCAA may show synergistic effects. The antitumor effects of sorafenib are due to its antiproliferative and antiangiogenic activities, including inhibiting the serine-threonine kinases Raf-1 and B-Raf, and the tyrosine kinases of vascular endothelial growth factor receptors 1–3 and platelet-derived growth factor receptor-β of tumor cells and vascular endothelial cells.[22] BCAA may regulate these signal transductions and enhance the antitumor activity of sorafenib by cross-talk mechanisms between sorafenib- and BCAA-mediated cellular signaling pathways. Additional studies are needed to determine the mechanism by which BCAA enhances the antitumor effects of sorafenib.

As for the prognosis of the 17 patients (12 Child–Pugh A patients and five Child–Pugh B patients), who were treated with BCAA granules in only a portion of sorafenib treatment and excluded in this study, the cumulative overall survival rate in this "partial BCAA group" was not different compared with that in BCAA and non-BCAA groups (data not shown) because of a small number of the partial BCAA group patients.

Sorafenib treatment is not generally recommended in the Child–Pugh B status. Our study included 40 Child–Pugh B patients who underwent sorafenib treatment, the Child–Pugh scores of whom were 7 points in 34 patients and 8 points or more in six patients. Sorafenib treatment should be carefully performed in Child–Pugh B patients in view of the clinical benefit and risk. In addition, our study revealed that the favorable effect of BCAA supplementation on the prognosis was not exhibited in the case of sorafenib treatment for Child–Pugh B patients.

In conclusion, our findings indicate that BCAA supplementation may be a valuable adjunctive therapy in sorafenib-treated patients with advanced HCC and Child–Pugh A status. Large-scale prospective studies are needed to determine whether BCAA enhances prognosis in these patients.

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## SUPPORTING INFORMATION

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

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HEPATOLOGY

# Geographic distribution and characteristics of genotype A hepatitis B virus infection in acute and chronic hepatitis B patients in Japan

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**Key words**
acute hepatitis B, chronic hepatitis B, distribution, genotype, human immunodeficiency virus.

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## Abstract

**Background and Aims:** The prevalence of sexually transmitted acute infections of the genotype A hepatitis B virus (HBV) has been increasing in Japan. Genotype A HBV is associated with an increased risk of HBV progression to chronic infection after acute hepatitis B (AHB) in adults. A nationwide survey was conducted to evaluate the geographic distribution, clinical, and virologic characteristics of genotype A AHB and chronic hepatitis B (CHB) in Japan.

**Methods:** Five hundred seventy AHB patients were recruited between 2005 and 2010, and 3682 CHB patients were recruited between 2010 and 2011. HBV genotypes were determined for 552 and 3619 AHB and CHB patients, respectively. Clinical characteristics were compared among different genotypes in AHB and CHB patients. Genomic characteristics of HBV genotype A were examined by molecular evolutionary analysis.

**Results:** Hepatitis B virus genotype A was the predominant genotype for AHB between 2005 and 2010. Phylogenetic analysis showed that all strains in the AHB patients with genotype A were classified into subtype Ae. Among CHB patients, the occurrence of genotype A was 4.1%, and genotype A was spreading in young adults. In genotype A CHB patients, early stage liver diseases were predominant, although liver diseases progressed to cirrhosis or hepatocellular carcinoma in some patients.

**Conclusions:** The distribution of HBV genotypes is quite different between AHB and CHB in Japanese patients. Genotype A infection is spreading in young adults of Japanese CHB patients. Sequences derived from Japanese AHB patients were identical to or closely resembled the sequences derived from other Japanese AHB patients.