A New Therapeutic Assessment Score for Advanced Hepatocellular Carcinoma Patients Receiving Hepatic Arterial Infusion Chemotherapy

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Abstract

Background & Aims
Hepatic arterial infusion chemotherapy (HAIC) is an option for treating advanced hepatocellular carcinoma (HCC). Because of the poor prognosis in HAIC non-responders, it is important to identify patients who may benefit from continuous HAIC treatment; however, there are currently no therapeutic assessment scores for this identification. Therefore, we aimed to establish a new therapeutic assessment score for such patients.

Methods
We retrospectively analyzed 90 advanced HCC patients with elevated baseline alpha-fetoprotein (AFP) and/or des-gamma-carboxy prothrombin (DCP) levels and analyzed various parameters for their possible use as predictors of response and survival. AFP and DCP responses were assessed after half a course of HAIC (2 weeks); a positive-response was defined as a reduction of ≥ 20% from baseline.

Results
Multivariate analysis identified DCP response (odds ratio 16.03, p < 0.001) as an independent predictor of treatment response. In multivariate analysis, Child-Pugh class A (hazard ratio [HR] 1.99, p = 0.018), AFP response (HR 2.17, p = 0.007), and DCP response (HR 1.90, p = 0.030) were independent prognostic predictors. We developed an Assessment for Continuous Treatment with HAIC (ACTH) score, including the above 3 factors, which ranged from 0 to 3. Patients stratified into two groups according to this score showed
significantly different prognoses (≤1 vs. ≥2 points: median survival time, 15.1 vs. 8.7 months; \( p = 0.003 \)).

**Conclusions**

The ACTH score may be useful in the therapeutic assessment of HCC patients receiving HAIC.

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**Introduction**

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second leading cause of cancer-related deaths worldwide [1]. Recent advances in treatment techniques, including hepatic resection, percutaneous ethanol injection (PEI), radiofrequency ablation (RFA), transcatheter arterial chemoembolization (TACE), hepatic arterial infusion chemotherapy (HAIC), sorafenib administration, and transplantation, have improved the prognosis of this malignancy [2–7]. However, the prognosis of advanced HCC patients, especially in the presence of vascular invasion and/or extrahepatic spread, remains poor.

For patients with advanced HCC, the multikinase inhibitor sorafenib is recommended as the current standard of care [8]. In contrast, HAIC is one of the recommended treatments in Japan [9]. However, there are no established criteria for the selection of HAIC or sorafenib in the treatment of advanced HCC. In addition, no randomized controlled trials have been conducted to compare these treatments. Although sorafenib has been shown to improve survival in advanced HCC patients with preserved liver function, the response rate of sorafenib therapy is low, at approximately 2–3% [7, 10]. In contrast, the response rate for HAIC therapy is approximately 30–40%, and survival is significantly longer in HAIC responders than in HAIC non-responders [11–13]. The median survival time (MST) is longer in patients who undergo HAIC using low-dose FP (cisplatin and 5-fluorouracil; 14.0 months) than in patients who do not receive active therapy (5.2 months; \( p < 0.0001 \)) [14]. Previously, our retrospective study of HAIC demonstrated a response rate of 36% and an MST of 10.2 months, with significantly longer survival in responders (MST in responders, 17.8 months; non-responders, 7.2 months; \( p < 0.0001 \)) [15]. Because of the poor prognosis of HAIC non-responders, it is important to identify patients who may benefit from continuous HAIC treatment. Although staging systems for HCC can predict patient prognosis [16, 17], there are currently no therapeutic assessment scores to aid decision-making with regard to continuous HAIC treatment. Therefore, we aimed to establish a new therapeutic assessment score for such patients.

**Patients and Methods**

**Patients**

Between July 1997 and July 2012, HAIC based on low-dose FP was administered to 130 patients admitted to our hospital with unresectable HCC. HCC was considered unresectable in cases of bilobar disease, extrahepatic metastasis, portal vein tumor thrombosis (PVTT), or locally advanced disease that was too extensive for resection. A diagnosis of HCC was based on imaging results and on elevated serum levels of alpha-fetoprotein (AFP) and/or des-gamma-carboxy prothrombin (DCP). Of the 130 patients, 90 patients with elevated baseline levels of AFP (≥20 ng/mL) and/or elevated baseline levels of DCP (≥40 mAU/mL) were enrolled in this retrospective cohort study. This study (H24-31) was approved by the Institutional Review Board...
Board of Yamaguchi University Hospital, and written informed consent was obtained from all patients before inclusion in the study. The study protocol was conducted according to the principles of the 1975 Declaration of Helsinki.

**Tumor Stage and PVTT Grading**

Tumor staging (T factor) was performed according to the Liver Cancer Study Group of Japan criteria, and based on whether the tumor was (1) solitary, (2) no greater than 2 cm in diameter, and (3) without vascular invasion. Stage I was defined as fulfilling all three conditions (T1), stage II as fulfilling two of the three conditions (T2), stage III as fulfilling one of the three conditions (T3), stage IV-A as fulfilling none of the three conditions (T4) with no distant metastasis or any T factor with lymph node metastasis, and stage IV-B as any T factor with distant metastases.

PVTT grading and hepatic vein tumor invasion grading were also assessed according to the criteria of the Liver Cancer Study Group of Japan [18, 19]. PVTT grading was based on the location of the tumor thrombus in the peripheral portal vein: Vp1, tumor thrombus in a third or more of the peripheral branches of the portal vein; Vp2, tumor thrombus in a second branch of the portal vein; Vp3, tumor thrombus in the first branch of the portal vein; or Vp4, tumor thrombus in the trunk of the portal vein. Hepatic vein tumor invasion grading was based on the location of the tumor thrombus in the hepatic vein: Vv1, tumor thrombus in any peripheral branch of the hepatic vein; Vv2, tumor thrombus in the posterior inferior hepatic vein trunk, short hepatic vein, or right, middle, or left hepatic vein trunk; or Vv3, tumor thrombus in the inferior vena cava.

**Catheter Placement**

A heparin-coated 5-French catheter (Anthron P-U Catheter; Toray Medical Co. Ltd., Tokyo, Japan), connected to a subcutaneously implanted reservoir, was inserted intraluminally from the femoral, subclavian, or brachial artery and was positioned in the proper or common hepatic artery. The gastroduodenal and right gastric arteries were occluded with steel coils to prevent gastroduodenal injury from the anticancer agents. The entire procedure was performed under local anesthesia. The reservoir device was filled with 5 mL (5000 units) of heparin solution every 2 weeks to prevent arterial occlusion.

**Chemotherapeutic Regimen**

The patients received repeated arterial infusion of chemotherapeutic agents via the injection port. Patients were treated with either HAIC using low-dose FP [20, 21], HAIC using low-dose FP with isovorin [22], HAIC combination therapy consisting of low-dose FP with isovorin and subcutaneous interferon (IFN)-alpha 2b [23], or HAIC combination therapy consisting of low-dose FP with isovorin and subcutaneous pegylated-interferon (PEG-IFN)-alpha-2b [24]. We previously reported no significant differences in response and survival between these four regimens [15].

One course of chemotherapy consisted of 5 consecutive days (days 1–5) of daily cisplatin administration (10 mg/body; Nippon Kayaku Co., Tokyo, Japan), followed by 5-fluorouracil (250 mg/body; Kyowa Hakko Co., Tokyo, Japan), Isovorn (6.25 mg/body; Wyeth KK, Tokyo, Japan or Kyowa, Hakko Kirin Co., Tokyo Japan) was administered daily on days 1–5. IFN-alpha-2b (3 MUD/day; Intron A, Schering-Plough KK, Osaka, Japan) was administered on days 1, 3, and 5, and PEG-IFN-alpha-2b (50 μg; PegIntron, Schering-Plough KK) was administered on day 1. Days 6 and 7 were rest days. This course was repeated for 2 weeks, suspended for 1 week, and then repeated again for 2 weeks. In one treatment cycle, arterial infusion...
chemotherapy was administered 20 times, IFN-alpha-2b was administered 12 times, and PEG-IFN-alpha-2b was administered 4 times. Both cisplatin and 5-fluorouracil were administered using a mechanical infusion pump set at 1 h and 5 h, respectively. Isovorin was administered at 10 min. The serotonin antagonist ondansetron hydrochloride (4 mg; Zofran, GlaxoSmithKline, Tokyo, Japan) was administered intravenously as an antiemetic agent.

Evaluation of Treatment Response

The evaluation of the response to treatment was classified according to the RECIST guidelines ver.1.1 [25]. Dynamic computed tomography or magnetic resonance imaging was performed before and after one course of HAIC. Complete response (CR) was defined as the disappearance of all target lesions. Partial response (PR) was defined as a decrease of at least 30% in the sum of the longest diameter of the target lesions compared to the pretreatment reference value. Progressive disease (PD) was defined as an increase of at least 20% in the sum of the longest diameter of the target lesions. Treatment response was classified as stable disease (SD) if none of these criteria were met. Patients who had not completed the first cycle of therapy were regarded as having PD if radiological disease progression was confirmed at that time.

AFP and DCP serum levels were measured using the LiBASys automated immunologic analyzer (Wako Pure Chemical Industries Ltd., Osaka, Japan). Both were obtained at baseline (prior to the administration of HAIC), 2 weeks after initiation of HAIC (half course of HAIC), and at the end of one course of HAIC (full course of HAIC). An AFP positive-response was defined as a reduction in serum AFP of more than 20% from baseline after half a course of HAIC [26, 27]. Similarly, a DCP positive-response was defined as a reduction in serum DCP of more than 20% from baseline after half a course of HAIC. A non-response was defined as a reduction of <20% from baseline and an increase (into the abnormal range) from the baseline of AFP or DCP level after half a course of HAIC.

Statistical Analysis

The data are expressed as the mean ± standard deviation. Univariate and multivariate analyses of the predictors of response were assessed by logistic regression analysis. We assessed 13 variables in the response, including gender (male or female), age (younger or older than 65 years), the presence of anti-hepatitis C virus antibody, hepatic reserve capacity (Child-Pugh score A or B), extrahepatic metastasis (yes or no), tumor stage (stage II-III or IV), previous treatments (yes or no), PVTT grade (Vp 0–2 or Vp3-4), inferior vena cava invasion (yes or no), AFP level (<1000 or ≥ 1000 ng/mL), DCP level (<1000 or ≥ 1000 mAU/mL), AFP response (yes or no), and DCP response (yes or no). Overall survival was calculated using the Kaplan–Meier method. The clinical data were assessed as predictors of survival using univariate and multivariate Cox proportional hazard regression analysis. Survival time was defined as the interval between the first HAIC and the last follow-up or death. The follow-up period ended on December 31, 2013. Statistical significance was defined as a p-value < 0.05. All analyses were performed using the JMP ver. 10.0 software package (SAS Institute, Cary, NC, USA).

Results

Patient Characteristics

The clinical profiles of the 90 HCC patients treated with HAIC are summarized in Table 1 and S1 Table. The patient population consisted of 75 men and 15 women, with a mean age of 65.7 years (range, 44–85 years). Fifty-eight patients tested positive for the anti-hepatitis C virus antibody, and 61 patients had previously undergone therapy for HCC. The median AFP and DCP
levels were 352.9 ng/mL (range, 0.9–274100 ng/mL) and 1029.0 mAU/mL (range, 6.0–344740 mAU/mL), respectively. Among 90 patients, 8 (8.9%), 19 (21.1%), and 63 (70.0%) had an elevated level of AFP, elevated level of DCP, or elevated level of both these tumor markers, respectively.

Of the 90 patients, 2 were treated with HAIC using low-dose FP, 62 were treated with HAIC using low-dose FP and isovorin, 15 were treated with combination therapy consisting of low-dose FP, isovorin, and subcutaneous IFN-alpha-2b, and 11 were treated with combination therapy consisting of low-dose FP, isovorin, and subcutaneous PEG-IFN-alpha-2b. The clinical observation period following completion of HAIC treatment ranged from 42–4597 days.

Response to Therapy and Predictors of Response

The response to therapy is summarized in Table 2. Of the 90 HCC patients, 1 patient (1.1%) exhibited CR, 30 (33.3%) exhibited PR, 39 (43.3%) exhibited SD, and 20 (20.2%) exhibited PD. The response rate, calculated as the number of patients exhibiting CR or PR divided by the total number of patients, was 34.4%.

With respect to AFP levels, among the 71 patients, the treatment response rate was 51.9% among AFP responders and 20.5% among AFP non-responders (p = 0.023). There were no significant differences in clinical characteristics between AFP responders and AFP non-responders (S2 Table). With respect to DCP levels, among the 82 patients, the treatment response rate was 56.4% in DCP responders and 14.0% in DCP non-responders (p < 0.001). There were no significant differences in clinical characteristics between DCP responders and DCP non-responders (S3 Table).

Predictors of response to therapy are shown in Table 3. Of the 13 factors analyzed by univariate analysis, two factors were significant predictors for response: AFP response (p = 0.006) and DCP response (p < 0.001). Multiple logistic regression analysis identified DCP response
odds ratio [OR] 16.03; 95% confidence interval [CI] 3.76–112.53; \( p < 0.001 \) as an independent predictor of response to therapy.

### Patient Survival and Predictors of Survival

The overall survival rates at 1, 2, and 3 years were 46.7%, 18.9%, and 8.9%, respectively (Fig 1). The MST was 10.6 months. Predictors of survival are shown in Table 4. Of the 13 factors analyzed using univariate analysis, two factors were significant predictors of survival: AFP response \( (p = 0.003) \) and DCP response \( (p = 0.017) \). AFP and DCP responders showed significantly longer overall survival than non-responders (AFP: MST, 17.4 vs. 8.2 months; DCP: MST, 15.0 vs. 9.3 months; Fig 2). In multivariate analysis, Child-Pugh score A (hazard

### Table 2. Response to therapy.

|                  | CR  | PR  | SD  | PD  | Response rate |
|------------------|-----|-----|-----|-----|---------------|
| **Total case (N = 90)** |     |     |     |     | 34.40%        |
| AFP responders (N = 27) | 1   | 13  | 10  | 3   | 51.90%        |
| AFP non-responders (N = 44) | 0   | 9   | 20  | 15  | 20.50%        |
| **Total (N = 71)** | 1   | 22  | 30  | 18  | 32.40%        |
| **DCP responders (N = 39)** | 1   | 21  | 11  | 6   | 56.40%        |
| DCP non-responders (N = 43) | 0   | 6   | 25  | 12  | 14.00%        |
| **Total (N = 82)** | 1   | 27  | 36  | 18  | 34.20%        |

*Responders versus non-responders.

AFP, alpha-fetoprotein; DCP, des-gamma-carboxyprothrombin

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease

### Table 3. Predictors of response to therapy.

| Factors                | Univariate analysis | Multivariate analysis |
|------------------------|---------------------|-----------------------|
|                        | \( P \) value       | Odds ratio           |
|                        |                     | 95% CI                |
|                        |                     | \( P \) value         |
| Age                    | 0.47                |                       |
| Sex                    | 0.623               |                       |
| Etiology               | 0.345               |                       |
| Child-Pugh score       | 0.608               |                       |
| Stage \(^a\)           | 0.263               |                       |
| Metastases             | 0.637               |                       |
| Pretreatment           | 0.149               |                       |
| Vp                     | 0.206               |                       |
| Vv                     | 0.887               |                       |
| AFP                    | 0.786               |                       |
| DCP                    | 0.779               |                       |
| AFP responder          | 0.006               | 3.53                  |
|                        |                     | 0.97–13.80            |
|                          |                     | 0.055                 |
| DCP responder          | \( < 0.001 \)       | 16.03                 |
|                        |                     | 3.76–112.53           |
|                          |                     | \( < 0.001 \)         |

AFP, alpha-fetoprotein; DCP, des-gamma-carboxyprothrombin; Vp, portal tumor thrombosis; Vv, hepatic vein tumor thrombosis

\(^a\) According to the Liver Cancer Study Group of Japan

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Fig 1. Cumulative survival of 90 advanced hepatocellular carcinoma patients receiving hepatic arterial infusion chemotherapy. The survival rates at 1, 2, and 3 years were 46.7%, 18.9%, and 8.9%, respectively. The median survival time (MST) was 10.6 months.

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Table 4. Predictors of survival after HAIC treatment.

| Factors          | Univariate analysis | Multivariate analysis |
|------------------|---------------------|-----------------------|
|                  | Hazard ratio | 95% CI | P value | Hazard ratio | 95% CI | P value |
| Age              | 1.26        | 0.82–1.98 | 0.294   |              |         |        |
| Sex              | 1.11        | 0.65–2.02 | 0.708   |              |         |        |
| Etiology         | 0.73        | 0.47–1.15 | 0.176   |              |         |        |
| Child-Pugh score | 1.49        | 0.97–2.29 | 0.07    | 1.99        | 1.13–3.51 | 0.018   |
| Stage a          | 1.25        | 0.80–2.00 | 0.337   | 1.25        | 0.71–1.94 | 0.479   |
| Metastases       | 1.2         | 0.71–1.94 | 0.479   |              |         |        |
| Pretreatment     | 0.95        | 0.60–1.56 | 0.846   |              |         |        |
| Vp               | 1.03        | 0.67–1.59 | 0.895   |              |         |        |
| Vv               | 0.67        | 0.31–1.24 | 0.208   |              |         |        |
| AFP              | 1.32        | 0.86–2.08 | 0.209   |              |         |        |
| DCP              | 0.97        | 0.63–1.50 | 0.904   |              |         |        |
| AFP responder    | 2.13        | 1.29–3.62 | 0.003   | 2.17        | 1.23–3.92 | 0.007   |
| DCP responder    | 1.74        | 1.10–2.74 | 0.017   | 1.9         | 1.06–3.42 | 0.03    |

AFP, alpha-fetoprotein; DCP, des-gamma-carboxyprothrombin; Vp, portal tumor thrombosis; Vv, hepatic vein tumor thrombosis

* According to the Liver Cancer Study Group of Japan

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ratio (HR) 1.99; 95% CI 1.13–3.51; \( p = 0.018 \), AFP response (HR 2.17; 95% CI 1.23–3.92; \( p = 0.007 \)), and DCP response (HR 1.90, 95% CI 1.06–3.42; \( p = 0.030 \)) were independent prognostic predictors (Table 4).

We developed an Assessment for Continuous Treatment with HAIC (ACTH) score based on the HR of the significant variables obtained from multivariate analysis, ranging from 0 to 3. The ACTH score consists of 3 parameters: Child-Pugh score, AFP response, and DCP response, and is calculated as follows: Child-Pugh score (A = 0, B = 1), AFP response (yes = 0, no = 1), and DCP response (yes = 0, no = 1) (Fig 3). Consequently, a change in the normal range of AFP and DCP between baseline and half a course of HAIC is given a score of 0.

**The ACTH Score Predicts Overall Survival**

Patients stratified into two groups according to their ACTH score; 46 (51.1%) and 44 patients (48.9%) were classified as having an ACTH score \( \leq 1 \) and \( \geq 2 \), respectively. These two groups showed significantly different prognoses (\( \leq 1 \) vs. \( \geq 2 \) points: MST, 15.1 vs. 8.7 months; \( p = 0.003 \); Fig 3).

**Discussion**

The prognosis of advanced HCC, especially in the presence of vascular invasion and/or extrahepatic spread, remains poor. In patients with advanced HCC who did not receive active therapy, the MST is 6–7 months [28]. In Japan, HAIC and sorafenib are the recommended treatments for advanced HCC [9]. However, there are no established criteria to determine which of these would be the most appropriate. Since 1997, we have been performing HAIC based on low-dose FP in advanced HCC patients [15, 20, 22–24, 29], as it has been shown that HAIC substantially prolongs survival in patients who achieve CR or PR. However, it has been shown that current therapies, worsen the outcome of HAIC refractory patients [15], with the exception of sorafenib, which may improve prognosis [30]. It is therefore important to evaluate the response to HAIC early in the treatment period. Until recently, we have assessed the
If the response to HAIC can be evaluated during treatment, we can switch HAIC refractory patients to alternate treatments such as sorafenib or new therapy within a clinical trial early in the treatment period. Recently, many staging systems for HCC that can predict patient prognosis, such as those developed by the Barcelona Clinic Liver Cancer (BCLC) [31], the Cancer of the Liver Italian Program (CLIP) [32], and the Groupe d’Etude et de Traitement du Carcinome Hpatocellulaire (GETCH) [33], as well as the Chinese University Prognostic Index (CUPI) [34], and the Japan Integrated Staging (JIS) [19], have been reported. However, these staging systems are not therapeutic assessment scores, and there are currently no therapeutic assessment scores to aid decision-making with regard to continuous HAIC treatment. Therefore, we developed a new therapeutic assessment score for advanced HCC patients receiving HAIC.

AFP and DCP are used in the diagnosis and surveillance of HCC [35, 36]. However, there is yet no consensus as to the usefulness of changes in tumor markers for assessing the response to HCC treatment [37]. It has been reported that changes in AFP levels after systemic therapy [38], locoregional therapy [39], and HAIC [26] may be predictive of treatment outcome. Therefore, we added two factors related to changes between pre-treatment and the end of a half-course of HAIC (AFP and DCP responses) to 11 pre-treatment factors. While a previous study assessed changes in tumor marker levels at the completion of two cycles of HAIC [26], no clinical studies have examined AFP and DCP responses at the mid-cycle of HAIC. We...
found that there was a significant correlation between the ratio of AFP/DCP after half a course of HAIC and after one complete course of HAIC compared to baseline (AFP: $r = 0.583$, $p < 0.001$, DCP: $r = 0.796$, $p < 0.001$; S1 Fig). These findings show that the AFP and DCP levels at the end of half a course of HAIC reflect those after a full course of HAIC.

For predictors of response, the univariate analysis showed that AFP response and DCP response were independent parameters, and the multivariate analysis further identified DCP response (OR, 16.03; 95% CI, 3.76–112.53; $p < 0.001$). The mean half-life of DCP is shorter than that of AFP (DCP, 3.2 days; AFP, 6 days) [40]. Thus, changes in DCP may be more useful than changes in AFP for assessing the early treatment response to HAIC.

We also assessed whether these factors could predict survival. The univariate analysis showed that AFP response and DCP response were independent parameters, and the multivariate analysis further identified Child-Pugh score (HR, 1.99; 95% CI, 1.13–3.51; $p = 0.018$), AFP response (HR, 2.17; 95% CI, 1.23–3.92; $p = 0.007$), and DCP response (HR, 1.90; 95% CI, 1.06–3.42; $p = 0.030$). Although the Child-Pugh score has previously been identified as a significant prognostic factor in many reports [5, 13, 21], only a few of these reports related to changes in tumor marker levels [26, 41].

Therefore, we developed the ACTH score based on the HR of the significant variables identified in multivariate analysis. The ACTH score consists of 3 parameters: Child-Pugh score, AFP response, and DCP response. AFP and DCP have been used as complementary tumor markers [35, 42]. Elevated levels of AFP or DCP were associated with malignant potential such...
as vascular invasion, tumor differentiation, and intrahepatic metastases, but they have different relationships with a number of clinicopathological variables of HCC [42]. Although HCC patients with either elevated AFP or elevated DCP before HAIC were included in this study, we defined a non-response with respect to these markers both as a reduction of <20% from baseline and an increase from baseline after half a course of HAIC. In addition, a change in the normal range of AFP and DCP between baseline and half a course of HAIC was defined as score 0. Toyoda, et al. reported that the number of elevated post-treatment tumor markers was significantly associated with survival in HCC patients [43], supporting this approach. The ACTH score identified two distinct groups with different prognoses (ACTH score ≤ 1 vs. ≥ 2 points: MST, 15.1 vs. 8.7 months; \( p = 0.003 \); Fig 3). When we analyzed 63 patients with elevated levels of both AFP and DCP, and stratified them into two groups according to this score, there was a significantly different prognosis between the groups (ACTH score ≤ 1 vs. ≥ 2 points: MST, 17.3 vs. 6.7 months; \( p = 0.001 \)) (S2 Fig).

For patients with a score ≤ 1, HAIC treatment would be continued, and for patients with a score ≥ 2, a second line therapy such as sorafenib and/or participation in a new clinical trial would be a better option (Fig 4).

There are limitations to this study. First, the chemotherapeutic regimens used were not uniform between all patients, even though all the regimens were based on low-dose FP. However, it was previously shown that there are no significant differences in response or survival between these regimens [29]. A validation study is required in HCC patients treated with a uniform low-dose FP regimen or other HAIC regimens. Second, this was a retrospective cohort study examining a small population. However, there have only been a few studies of HAIC in more than 100 advanced HCC patients [11–13, 29, 44, 45]. A prospective study of a larger patient population is necessary. Third, this score cannot be used in patients with normal levels of tumor markers. However, patients with high tumor marker levels tend to have advanced HCC, especially if there is also vascular invasion and/or extrahepatic spread [46]; indeed, 90 of the 130 patients (69%) could be enrolled in this study.

In conclusion, the ACTH score, which consists of 3 simple factors, may help in the therapeutic assessment of HCC patients receiving HAIC, and could make it possible to use a new treatment earlier for non-responders. This may in turn significantly improve patient survival.

Supporting Information

S1 Fig. Correlation between the AFP:DCP ratio at base line and after half a course of HAIC. There was a significant correlation between the AFP:DCP ratio at baseline and after half a course of HAIC. (AFP: \( r = 0.583 \); \( p < 0.001 \), DCP: \( r = 0.796 \); \( p < 0.001 \)). (TIF)

S2 Fig. The ACTH score predicts overall survival in advanced hepatocellular carcinoma patients with AFP and DCP. When we analyzed 63 patients with elevated levels of both AFP and DCP, and stratified into two groups according to this score, there was a significantly different prognosis between the groups (ACTH score ≤ 1 vs. ≥ 2 points: MST, 17.3 vs. 6.7 months; \( p = 0.001 \)). (TIF)

S1 Table. Patient Characteristics. (TIF)

S2 Table. Characteristics of patients with elevated AFP at baseline. (TIF)
S3 Table. Characteristics of patients with elevated DCP at baseline.

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Author Contributions
Conceived and designed the experiments: I. Saeki TY. Performed the experiments: I. Saeki NT T. Iwamoto TM YU IH T. Ishikawa TT KU. Analyzed the data: I. Saeki TY NT T. Iwamoto TM YU IH T. Ishikawa TT KU. Contributed reagents/materials/analysis tools: TY NY ST I. Sakaida. Wrote the paper: I. Saeki TY.

References
1. GLOBOCAN. 2012. Available: http://globocan.iarc.fr/Default.aspx.
2. El-Serag H, Marrero J, Rudolph L, Reddy K. Diagnosis and treatment of hepatocellular carcinoma. Gastroenterology. 2008; 134(6):1752–63. doi: S0016-5085(08)00426-5 [pii] doi:10.1053/j.gastro.2008.02.090 PMID: 18471552.
3. Ebara M, Okabe S, Kita K, Sugiura N, Fukuda H, Yoshikawa M, et al. Percutaneous ethanol injection for small hepatocellular carcinoma: therapeutic efficacy based on 20-year observation. J Hepatol. 2005; 43(3):458–64. doi: S0168-8278(05)00381-8 [pii] doi:10.1016/j.jhep.2005.03.033 PMID: 16005538.
4. Matsui O, Kadoya M, Yoshikawa J, Gabata T, Araki K, Demachi H, et al. Small hepatocellular carcinoma: treatment with subsegmental transcatheter arterial embolization. Radiology. 1993; 188(1):79–83. PMID: 8390073.
5. Yamashita T, Kimura T, Kurokawa F, Aoyama K, Ishikawa T, Tajima K, et al. Prognostic factors in patients with advanced hepatocellular carcinoma receiving hepatic arterial infusion chemotherapy. J Gastroenterol. 2005; 40(1):70–8. doi: 10.1007/s00535-004-1494-7 PMID: 15692792.
6. Todo S, Furukawa H. Living donor liver transplantation for adult patients with hepatocellular carcinoma: experience in Japan. Ann Surg; 2004; 240(3):451–9; discussion 9–61. doi:00000658-200409000-00006 [pii] PMID: 15319716; PubMed Central PMCID: PMCPMC1356435.
7. Llovet J, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc J, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008; 359(4):378–90. PMID: 18650514, doi:10.1056/NEJMoa0708857
8. Bruix J, Sherman M, Practice Guidelines Committee AeAfSoLD. Management of hepatocellular carcinoma. Hepatology. 2005; 42(5):1208–36. doi: 10.1002/hep.20933 PMID: 16250051.
9. Ari S, Sata M, Sakamoto M, Shimada M, Kumada T, Shina S, et al. Management of hepatocellular carcinoma: Report of Consensus Meeting in the 45th Annual Meeting of the Japan Society of Hepatology (2009). Hepatol Res. 2010; 40(7):667–85. doi: 10.1111/j.1872-034X.2010.00673.x PMID: 20633193.
10. Cheng AL, Kang YK, Chen Z, Tsaio CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2009; 10(1):25–34. doi:10.1016/S1470-2045(08)70285-7
11. Obi S, Yoshida H, Toune R, Unuma T, Kanda M, Sato S, et al. Combination therapy of intraarterial 5-fluorouracil and systemic interferon-alpha for advanced hepatocellular carcinoma with portal venous invasion. Cancer. 2006; 106(9):1990–7. doi:10.1002/cncr.21832 PMID: 16565970.
12. Nagano H, Wada H, Kobayashi S, Marubashi S, Eguchi H, Tanemura M, et al. Long-term outcome of combined interferon-α and 5-fluorouracil treatment for advanced hepatocellular carcinoma with major portal vein thrombosis. Oncology. 2011; 80(1–2):63–9. doi:10.1159/000328281 PMID: 21659784.
13. Miyaki D, Aikata H, Honda Y, Naeshiro N, Nakahara T, Tanaka M, et al. Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma according to Child-Pugh classification. J Gastroenterol Hepatol. 2012; 27(12):1850–7. doi: 10.1111/j.1440-1746.2012.07276.x PMID: 23020312.
14. Nousou K, Miyahara K, Uchida D, Kuwaki K, Izumi N, Omata M, et al. Effect of hepatic arterial infusion chemotherapy of 5-fluorouracil and cisplatin for advanced hepatocellular carcinoma in the Nationwide Survey of Primary Liver Cancer in Japan. Br J Cancer. 2013; 109(7):1904–7. doi:10.1038/bjc.2013.542 PMID: 24008659; PubMed Central PMCID: PMCPMC3790188.
15. Yamasaki T, Sakaeda I. Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma and future treatments for the poor responders. Hepatol Res. 2012; 42(4):340–8. doi: 10.1111/j.1872-034X.2011.00938.x PMID: 22151009.

16. Shao YY, Lu LC, Lin ZZ, Hsu C, Shen YC, Hsu CH, et al. Prognosis of advanced hepatocellular carcinoma patients enrolled in clinical trials can be classified by current staging systems. Br J Cancer. 2012; 107(10):1672–7. doi: 10.1038/bjc.2012.466 PMID: 23059748; PubMed Central PMCID: PMCPMC3493875.

17. Huitzil-Melendez FD, Caparu M, O'Reilly EM, Duffy A, Gansukh B, Saltz LL, et al. Advanced hepatocellular carcinoma: which staging systems best predict prognosis? J Clin Oncol. 2010; 28(17):2889–95. doi: 10.1200/JCO.2009.25.9895 PMID: 20458042; PubMed Central PMCID: PMCPMC3651603.

18. Japan LCSGo. The General Rules for the Clinical and Pathological Study of Primary Liver Cancer. 5th edn ed. Tokyo2009.

19. Kudo M, Chung H, Osaki Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new integrated staging system, the Japan Integrated Staging Score (JIS score). J Gastroenterol. 2003; 38(3):207–15. doi: 10.1007/s005350300038 PMID: 12673442.

20. Yamasaki T, Kurokawa F, Shirahashi H, Kusano N, Hironaka K, Masuhara M, et al. Novel arterial infusion chemotherapy using cisplatin, 5-fluorouracil, and leucovorin for patients with advanced hepatocellular carcinoma. Hepatol Res. 2002; 23(1):7–17. doi: S1386634601001632 [pii]. PMID: 12084550.

21. Ando E, Tanaka M, Yamashita F, Kuromatsu R, Yutani S, Fukumori K, et al. Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis: analysis of 48 cases. Cancer. 2002; 95(3):588–95. doi: 10.1002/cncr.10694 PMID: 12209752.

22. Yamasaki T, Kurokawa F, Takami T, Omori K, Kawaguchi K, Tsuchiya M, et al. Arterial infusion chemotherapy using cisplatin, 5-fluorouracil, and isofoxin for patients with advanced hepatocellular carcinoma, pilot study: Is a high dose of the biochemical modulator effective? Hepatol Res. 2003; 27(1):36–44. doi: S138663460301955 [pii]. PMID: 12957205.

23. Takaki-Hamabe S, Yamasaki T, Saeki I, Harima Y, Okita K, Terai S, et al. Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma: Is the addition of subcutaneous interferon-alpha-2b beneficial? Hepatol Res. 2009; 39(3):223–30. doi: HEP458 [pii] doi: 10.1111/j.1872-034X.2008.00458.x PMID: 19054152.

24. Okita K, Yamasaki T, Hamabe T, Saeki I, Harima Y, Terai S, et al. Hepatic Arterial Infusion Chemotherapy in Combination with Pegylated Interferon-a-2b for Advanced Hepatocellular Carcinoma. Hepatogastroenterology. 2012; 59(114):533–7. doi: 10.5754/hepg1013 PMID: 22353519.

25. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009; 45(2):228–47. doi: 10.1016/j.ejca.2008.10.026 PMID: 19097774.

26. Lee MH, Kim SU, Kim dY, Ahn SH, Choi EH, Lee KH, et al. Early on-treatment predictions of clinical outcomes using alpha-fetoprotein and des-gamma-carboxy prothrombin responses in patients with advanced hepatocellular carcinoma. J Gastroenterol. 2010; 27(2):313–22. doi: 10.1111/j.1440-1746.2011.06867.x PMID: 21793906.

27. Chan SL, Mo FK, Johnson PJ, Hui EP, Ma BB, Ho WM, et al. New utility of an old marker: serial alpha-fetoprotein measurement in predicting radiologic response and survival of patients with hepatocellular carcinoma undergoing systemic chemotherapy. J Natl Cancer Inst. 2008; 100(10):698–711. doi: 10.1093/jnci/djn134 PMID: 18477802.

28. Urayama N, Yamasaki T, Harima Y, Saeki I, Zaitsu J, Hamabe S, et al. Hepatic arterial infusion chemotherapy in patients with advanced hepatocellular carcinoma: analysis of 114 cases (in Japanese with English abstract). Kanzo. 2011; 52(7):449–60.

29. Miyauchi D, Aikata H, Kan H, Fujino H, Urabe A, Masaki K, et al. Clinical outcome of sorafenib treatment in patients with advanced hepatocellular carcinoma refractory to hepatic arterial infusion chemotherapy. J Gastroenterol Hepatol. 2013; 28(12):1834–41. doi: 10.1111/jgh.12311 PMID: 23808713.

30. Llovet JM, Brú C, Brúix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis. 1999; 19(3):329–38. doi: 10.1055/s-2007-1007122 PMID: 10518312.

31. investigators. TCotLIPC. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology. 1999; 29(3):751–5. doi: 10.1002/hep.102083022 PMID: 9731568.

32. Chevet S, Trinchet JC, Mathieu D, Rached AA, Beaugrand M, Chastang C, A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. Groupe d’Etude et de Traitement du Carcinome Hépatocellulaire. J Hepatol. 1999; 31(1):133–41. PMID: 10424293.
34. Leung TW, Tang AM, Zee B, Lau WY, Lai PB, Leung KL, et al. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program staging system: a study based on 926 patients. Cancer. 2002; 94(6):1760–9. PMID: 11920539.

35. Aoyagi Y, Oguro M, Yanagi M, Mita Y, Suda T, Suzuki Y, et al. Clinical significance of simultaneous determinations of alpha-fetoprotein and des-gamma-carboxy prothrombin in monitoring recurrence in patients with hepatocellular carcinoma. Cancer. 1996; 77(9):1781–6. doi: 10.1002/(SICI)1097-0142(19960501)77:9<1781::AID-CNCR4>3.0.CO;2-F PMID: 8646674.

36. Mita Y, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H. The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. Cancer. 1998; 82(9):1643–8. PMID: 9576283.

37. Park H, Park JY. Clinical significance of AFP and PIVKA-II responses for monitoring treatment outcomes and predicting prognosis in patients with hepatocellular carcinoma. Biomed Res Int. 2013; 2013:310427. doi:10.1155/2013/310427 PMID: 24455683; PubMed Central PMCID: PMCPMC3885148.

38. Vora SR, Zheng H, Stadler ZK, Fuchs CS, Zhu AX. Serum alpha-fetoprotein response as a surrogate for clinical outcome in patients receiving systemic therapy for advanced hepatocellular carcinoma. Oncologist. 2009; 14(7):717–26. doi: 10.1634/theoncologist.2009-0038 PMID: 19581525.

39. Riaz A, Ryu RK, Kulik LM, Mulcahy MF, Lewandowski RJ, Minocha J, et al. Alpha-fetoprotein response after locoregional therapy for hepatocellular carcinoma: oncologic marker of radiologic response, progression, and survival. J Clin Oncol. 2009; 27(34):5734–42. doi: 10.1200/JCO.2009.23.1282 PMID: 19805671.

40. Kishi K, Sonomura T, Mitsuzane K, Nishida N, Kimura M, Satoh M, et al. Time courses of PIVKA-II and AFP levels after hepatic artery embolization and hepatic artery infusion against hepatocellular carcinoma: relation between the time course and tumor necrosis. Radiat Med. 1992; 10(5):189–95. PMID: 1279748.

41. Kim BK, Ahn SH, Seong JS, Park JY, Kim dY, Kim JK, et al. Early α-fetoprotein response as a predictor for clinical outcome after localized concurrent chemoradiotherapy for advanced hepatocellular carcinoma. Liver Int. 2011; 31(3):369–76. doi: 10.1111/j.1478-3231.2010.02368.x PMID: 21083802.

42. Yamamoto K, Imanura H, Matsuyama Y, Hasegawa K, Beck Y, Sugawara Y, et al. Significance of alpha-fetoprotein and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma undergoing hepatectomy. Ann Surg Oncol. 2009; 16(10):2795–804. doi: 10.1245/s10434-009-0618-y PMID: 19669841.

43. Toyoda H, Kumada T, Tada T, Niinomi T, Ito T, Kaneoka Y, et al. Prognostic significance of a combination of pre- and post-treatment tumor markers for hepatocellular carcinoma curatively treated with hepatectomy. J Hepatol. 2012; 57(6):1251–7. doi: 10.1016/j.jhep.2012.07.018 PMID: 22824818.

44. Kim BK, Park JY, Choi HJ, Kim dY, Ahn SH, Kim JK, et al. Long-term clinical outcomes of hepatic arterial infusion chemotherapy with cisplatin with or without 5-fluorouracil in locally advanced hepatocellular carcinoma. J Cancer Res Clin Oncol. 2011; 137(4):659–67. doi: 10.1007/s00432-010-0917-5 PMID: 20552225.

45. Yamashita T, Arai K, Sunagozaka H, Ueda T, Terashima T, Mizukoshi E, et al. Randomized, phase II study comparing interferon combined with hepatic arterial infusion of fluorouracil plus cisplatin and fluorouracil alone in patients with advanced hepatocellular carcinoma. Oncology. 2011; 81(5–6):281–90. doi: 10.1159/000343449 PMID: 22133996.

46. Zhou L, Liu J, Luo F. Serum tumor markers for detection of hepatocellular carcinoma. World J Gastroenterol. 2006; 12(8):1175–81. PMID: 16534867; PubMed Central PMCID: PMCPMC4124425.