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Prognostic Improvement of Patients with Advanced Liver Cancer after Active Hexose Correlated Compound (AHCC) Treatment

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SUMMARY Most patients with liver cancer are diagnosed when they are not suitable for resection. Although some palliative approaches can be applied to these patients, the overall survival rate remains unsatisfactory. Active hexose correlated compound (AHCC), a newly developed functional food, has been shown to act as a potent biological response modifier in *in vitro* experiments. Recently, AHCC was found to improve the prognosis of hepatocellular carcinoma patients following surgical treatment. We investigated whether AHCC could prolong survival and improve the prognosis of patients with advanced liver cancer. A prospective cohort study was performed with 44 patients with histologically confirmed liver cancer. All of the patients underwent supportive care. Survival time, quality of life, clinical and immunological parameters related to liver function, cellular immunity, and patient status were determined. Of the 44 patients, 34 and 10 received AHCC and placebo (control) orally, respectively. Patients in the AHCC treated-group had a significantly prolonged survival when compared to the control group by Mann-Whitney test (95% CI, p = 0.000). Quality of life in terms of mental stability, general physical health status, and ability to have normal activities were significantly improved after 3 months of AHCC treatment when tested using the Wilcoxon signed-rank test (on one-sided test, p = 0.028, 0.037, and 0.040, respectively). The apparent different clinical parameters between the two groups were the levels of albumin and percentage of lymphocytes with p-values of 0.000 and 0.026 at 1 and 2 months after treatment, respectively. Unlike the control patients, AHCC treated-patients with longer survival time had the tendency of better outcomes since the levels of AST and ALT had not increased rapidly from their baselines at follow-up. In addition, the levels of total IL-12 and neopterin were slightly increased in AHCC treated-patients. This study suggests that AHCC intake could prolong the survival and improve the prognosis of patients with advanced liver cancer and delay the gradual decline of their physiological status.

Liver cancer is the sixth most common cancer worldwide with widely varying incidences over different geographical areas. It is the third most common cause of cancer mortality.[1-3] Survival rates of primary liver cancer are uniformly poor in both high-rate and low-rate areas. The comparable mortality rates indicate very little difference in survival in the contrasting areas.[4] There are two main histologic types of primary liver cancer: hepatocellular carcinoma (HCC), a frequently occurring tumor accounting for 75-90% of liver cancers[5] and cholangiocarcinoma (CCA), a less common type, comprising 10-25% of all liver cancers, except for some regions which include areas endemic for liver flukes, *e.g.* Thailand.[1,3,6]

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Most patients with liver cancer are diagnosed at a late or advanced stage.[7,8] A significant proportion of patients is not offered any other therapy than the best supportive care. Moreover, many others are given treatments that cannot be considered more than suboptimal since they barely improve survival.[7,9] There have been many attempts to treat the cancer. One of these approaches aims to stimulate the patient's immune system by using biological response modifiers (BRMs).[10-13] Several investigators have isolated and purified immunomodulatory polysaccharides as BRMs from mushrooms because of their attraction as food and as source material for the development of drugs.[14] Recently, lentinan, schizophyllan and krestin have been accepted as immunoceuticals in several oriental countries.[15-16]

Active hexose correlated compound (AHCC) is a functional food developed by the Amino Up Chemical Co. Ltd (Sapporo, Japan) in 1989.[10,15,16] AHCC is an extract of *Basidiomycotina,* which is obtained through the hybridization of several types of mushrooms.[17] Up to 74% of AHCC components are oligosaccharides. Nearly 20% of the oligosaccharides have acetylated α-1,4-glucan structures and some esterized hydroxyl groups with a low molecular weight of approximately 5,000, which is orally bioavailable. AHCC also contains other polysaccharides including β-glucans. Both α- and β-glucans are thought to be responsible for the biological activities.[10,17,18] A wide range of biological, pharmacological and clinical potentials of AHCC has been investigated so far. As an immunoceutical included in the general category of nutraceuticals or dietary supplements, AHCC is well tolerated and largely free of adverse effects.[19-21] AHCC has been reported to suppress thymic apoptosis induced by dexamethasone,[21] and to decrease ferric nitrilotriacetate (Fe-NTA)-mediated excretion of 8-hydroxy-2′-deoxyguanoside (8-OHdG) in rat urine.[22] AHCC is considered as a potent BRM that enhances NK cell activity,[23] as well as the immune responses in cancer patients, *i.e.*, enhancement of IL-12 and IFNγ production, including enhancement of NK activity to normal levels.[24] More recently, AHCC treatment after surgical operation has been shown to improve the quality of life (QOL) and prognosis of patients with hepatocellular carcinoma.[10] AHCC can act as a potent antioxidant which protects against disorders induced by oxidative stress.[18] These results suggest that AHCC acts as a promising BRM, antioxidant, antimutagenic and/or anticarcinogenic agent. However, the precise mechanisms by which AHCC exerts its beneficial effects remain to be elucidated.

This study was initiated to evaluate the effects of AHCC, as an orally administered BRM, on the prognosis of patients with advanced liver cancer focusing on the time course effects of AHCC on cell-mediated immunity in terms of Th-1 cytokine production compared to other relevant clinical parameters.

MATERIALS AND METHODS

Patients and follow-up

To determine whether AHCC can improve the prognosis of patients with advanced liver cancer by oral administration, a prospective cohort study was performed. Forty-four patients with advanced liver cancer who registered at the National Cancer Institute, Bangkok, Thailand, from August 2002 to July 2004, were included in the study by the following criteria: (1) histologically proven advanced liver cancer, (2) the tumor was unresectable and unsuitable for chemoembolization, (3) the performance status grade following ECOG was not more than 2, (4) no brain metastases nor psychiatric illness, (5) the hemato-, hepato-, and renal functions were still adequate, and (6) there was at least a one-month treatment-free period after previous conventional therapy. Thirty four and 10 patients were randomized into AHCC-treated and placebo (control) groups, respectively. All enrolled patients received supportive treatment and signed an informed consent. The patients were trusted with the self-administration of AHCC or placebo by ingesting 6 grams daily until the end of their life.

Clinical parameters were examined monthly or as specified to evaluate the patients' status and to compare between the AHCC treated- and control groups. The clinical parameters included: (1) quality of life (QOL), (2) hematological parameters in EDTA blood: hemoglobin, lymphocyte and white blood cell counts, and platelet counts, (3) biochemical parameters in serum: albumin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase

(ALT), and alkaline phosphatase (ALP), and (4) immunological parameters in citrated plasma: Th-1 cytokines, *i.e.*, gamma-interferon (IFNγ) and interleukin-12 (IL-12), and a marker of macrophage activation, neopterin. Magnetic resonance imaging (MRI) was also performed on patients who survived for more than one year.

The study protocol conformed to ethical guidelines and was approved by the Scientific Committee of the National Cancer Institute and the Ministry of Public Health, Thailand.

Measurement of clinical parameters in blood samples

All hematological and biochemical parameters in the study were analyzed on the CELLDYN 3500 automated analyzer (ABBOTT laboratories, IL, USA) and on the Hitachi 917 automated analyzer (Roche Diagnostics Gmbh, Mannheim, Germany), respectively. IFNγ and total IL-12 were measured using ELISA kits from Pierce Biotechnology, IL, USA. Neopterin was analysed using the Neopterin ELISA kit (IBL, Harnburg, Germany). The absorbances of ELISA products were read on the BEP III (Dade Boehring, USA).

Measurement of QOL

Quality of life was evaluated using a QOL questionnaire containing 28 items with 5 rating answer choices to investigate the patients' appraisals of their current levels of functioning and satisfaction. Patients were invited to attend an interview session of the researcher explaining the purpose of the survey, encouraged to ask questions in order to aid their understanding, and were then asked to complete all questions on their own. The appraisals were performed before and after 3 months of treatment.

Statistical analysis

In order to evaluate the homogeneity of the AHCC treatment *versus* the control group with respect to pretreatment clinical factors, data were analyzed using the chi-square or Mann-Whitney test to compare differences between the two groups. The primary hypothesis of improved survival time in patients with advanced liver cancer after treatment was tested with the Kaplan-Meier survival curve. The ninety-five percent confidence intervals were calculated by Mann-Whitney test for the absolute difference in median survival time between AHCC treated- and control groups. Differences in the clinical parameters during follow-up between the two groups and at different time points were statistically tested with the Mann-Whitney test. The QOL questionnaire was extracted by the factor analysis method and statistically tested for sufficiency with the chi-square test. The differences of medians of the scores for the extracted factors between before and after AHCC treatment were analyzed by paired Wilcoxon signed-rank test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Oral AHCC showed no side effects since none of the patients in the AHCC group refused to continue the use of AHCC during the study. The clinical background of the patients in the AHCC treated- and control groups is shown in Table 1. The homogeneity of genders and types of liver cancer (HCC/CCA) between the two groups were tested using the chi-square test with p values of 0.561 and 0.826, respectively. Before treatment, there were no significant differences in the clinical background between the two groups confirmed by Mann-Whitney statistics except for total bilirubin of which the median level in the control group was significantly higher than in the AHCC group. However, the levels of alkaline phosphatase in both groups were similar as well as the raised bilirubin in both conjugated- and unconjugated-forms.

Overall survival

All patients with advanced liver cancer in the AHCC group were still alive by 1.5 months, whereas 5 (50%) patients in the control group had died. The follow-up period ranged from 1.5 to more than 24 months in the AHCC group, and from 0.5 to 3.5 months in the control group. In the AHCC treated-group, the interquartile interval of survival was 2.9-5.3 months whereas in the control it was 1.4-2.6 months. Patient survival was significantly higher ($p = 0.000$, Mann-Whitney test) in the AHCC group than in the control group with a median survival time of 3.5 months and 1.5 months, respectively (Fig. 1).

Table 1 Clinical background of patients in the AHCC treated- and control groups

	AHCC group	Control group	*p*-value
Patient characteristics	(N = 34)	(N =10)	
Age (years)	48.5 (34-72)	51.5 (37-71)	0.716
Gender (male/female)	28/6	9/1	0.561
HCC/CCA	25/9	7/3	0.826
HBsAg (positive)	14 of 27	6 of 9	-
Biochemical parameters			
Albumin (g/dl)	3.7 (3.4-4.0)	3.5 (2.9-3.8)	0.194
Total bilirubin (mg/dl)	0.8 (0.6-2.0)	2.7 (1.0-6.7)	0.027
Aspartate transaminase (U/l)	79 (48-173)	108 (57-154)	0.516
Alamine transaminase (U/l)	51 (30-117)	39 (29-53)	0.384
Alkaline phosphatase (U/l)	194 (126-340)	223 (167-303)	0.327
Hematological parameters			
Hemoglobin (g/dl)	12.5 (11.0-13.4)	11.6 (10.1-13.4)	0.385
Platelet counts ($x10^3/\mu l$)	242 (208-340)	214 (153-318)	0.449
WBC counts ($x10^3/\mu l$)	8.7 (6.4-10.8)	6.4 (4.8-9.8)	0.145
Lymphocytes ($x10^3/\mu l$)	1.86 (1.43-2.27)	1.42 (0.79-1.90)	0.101
Lymphocytes (%)	22 (17-30)	20(17-24)	0.401
Immunological parameters	(N = 24)	(N = 6)	
Total IL-12 (pg/ml)	27 (9-81)	40 (24-76)	0.432
	(N = 29)	(N = 7)	
Neopterin (nmol/l)	9 (5-14)	12 (8-31)	0.270

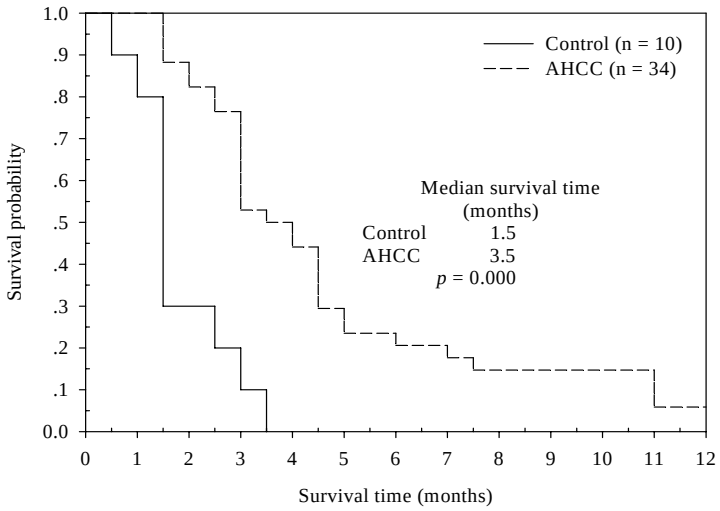

Fig. 1 Kaplan-Meier estimates of overall survival of patients. The solid line indicates survival in the control group, and the dashed line represents the AHCC treated-group. There was a significant difference in overall survival between the two groups ($p = 0.000$).

Quality of life (QOL)

Because of the mortality in the control group before the second QOL appraisal at 3 months of treatment, only QOL data from the 33 patients in the AHCC group could be analyzed. From the twenty-eight items of the QOL questionnaire, five factors were extracted by the factor analysis method. The hypothesis that five factors were sufficient was proven by chi-square statistics with a *p*-value of 0.0002. These five factors included: (1) acceptance of disease, (2) mental stability, (3) ability to help him/herself (defecation and urination), (4) general physical health status, and (5) ability to have normal activities. Table 2 demonstrates means, SD and median of the scores of these five factors before and after AHCC treatment. The means of factors 1, 2, 3 and 4 seemed to increase slightly from before to after treatment. However there was no significant difference when using the paired Wilcoxon signed-rank test except for factor 2 (mental stability), factor 4 (general physical health status), and factor 5 (ability to have normal activities) which were significantly different by the one-sided test with *p*-values of 0.028, 0.037, and 0.040, respectively. A paired t-test was not used for analysis since the scores of the five factors did not conform to normal distribution (Shapiro-Wilk normality test).

Clinical parameters

Ten biochemical and hematological parameters were investigated in the two groups. As half of the patients in the control group had died within 1.5 months, the median of these parameters of the two groups were compared at 0, 1, and 2 months as demonstrated in Figs. 2 and 3, and summarized in Tables 3 and 4, respectively. Of the ten parameters, two parameters including serum level of albumin, and percentage of lymphocytes in blood, were significantly higher in the AHCC treated-group than in the control group. The significant differences of these two parameters between the two groups could be noticed after one and two months of treatment with *p*-values of 0.000 and 0.026, respectively (Figs. 2A, 3E). The serum level of total bilirubin seemed to be lower in the AHCC treated-group, but this was not conclusive because a difference in total bilirubin was detected since the study had started. For other clinical parameters, however, there was a tendency of better results in the AHCC treated-group since the level of these parameters seemed not to increase as rapidly as in the control group. These parameters included serum levels of AST and ALT (Figs. 2C, 2D).

For immunological parameters, the levels of gamma-interferon (IFNγ) both in the AHCC treated- and control groups were lower than the test kit's minimum level of detection (< 2 pg/ml). The expected values from apparently healthy individuals as described in the manufacturer's instructions are only 0 to 2.6 pg/ml (n = 45). The plasma levels of interleukin-12 (IL-12) and neopterin before treatment were similar in both groups as shown in Table 1. Later on, there were not enough data of IL-12 and neopterin from the control group (n = 1) to be compared to those of the AHCC treated-group, so only the median IL-12 and neopterin levels from the AHCC treated-group were investigated until 3 months of treatment (Fig. 4 and Table 5). The me-

Table 2 Means, SD, and medians of the five factors analyzed from QOL questionnaires before and after AHCC treatment

Variable	Before						After						Two-sided *p*-value	One-sided *p*-value
	Obs[a]	NA[b]	Mean	SD	Median	Range	Obs[a]	NA[b]	Mean	SD	Median	Range		
Factor 1	27	6	3.54	0.98	3.86	1-5	28	3	3.77	0.78	3.93	1-57-5	0.350	0.175
Factor 2	30	3	3.54	0.94	3.75	1-5	28	3	3.67	0.69	3.75	1.5-4.75	0.057	0.028
Factor 3	32	1	3.02	1.19	3.00	1-5	31	0	3.27	0.95	3.00	1.25-5	0.305	0.153
Factor 4	32	1	3.47	1.30	3.67	1-5	30	1	3.82	1.26	4.33	1-5	0.073	0.037
Factor 5	32	1	4.17	1.06	4.5	1.33-5	29	2	4.06	1.17	4.67	1.33-5	0.080	0.040

Factor 1, acceptance of disease; Factor 2, mental stability; Factor 3, ability to help him/herself; Factor 4, general physical health status; Factor 5, ability to have normal activities; Obs[a], number of patients in observation; NA[b], number of patients with no answer

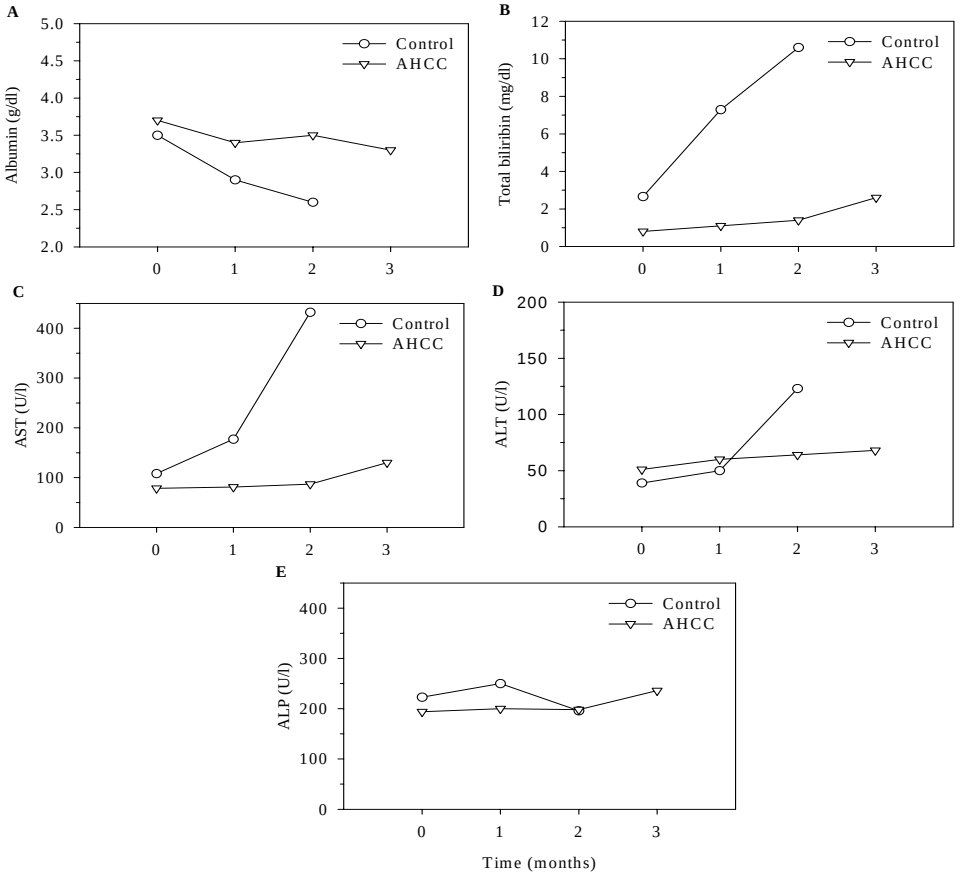

Fig. 2 The median profiles of biochemical parameters of patients. The open circles indicate the levels of the appropriate parameters in the control group, and the open triangles represent the levels in the AHCC treated-group. The medians of these parameters are: (A) albumin, (B) total bilirubin, (C) aspartate aminotransferase (AST), (D) alanine aminotransferase (ALT), and (E) alkaline phosphatase (ALP).

dian IL-12 level in AHCC treated-patients seemed to rise from 27 to 38 pg/ml during 3 months of follow-up. Expected values from apparently healthy individuals in our study ranged from 11 to 56 pg/ml (n = 24) with median and interquartile intervals of 33 and 22-44 pg/ml, respectively. The median neopterin level seemed not to change significantly ranging from 9 to 13 nmol/l. However, the expected value is less than 10 nmol/l.

Follow-up in long survival patients

Follow–up values of 6 interesting clinical parameters in 6 (18%) AHCC treated-patients with a survival time of more than 7 months (2 times of median survival) are shown in Fig. 5. These parameters included albumin, AST, ALT, lymphocytes (%), IL-12 and neopterin. The parameters of the individual profiles seemed not to change (decrease or increase) significantly compared to the median profiles of the control group. These results suggested that AHCC treated-patients with a longer survival could maintain their physiological parameters until the last 1 or 2 months of their life as seen in the albumin, lymphocytes (%), and neopterin parameters. In the patient who survived more than 24 months (AR4), all 6 parameters seemed not to change vitally, showing a good prognosis which correlated with the survival.

Fig. 6 shows photographs of erythematous skin (spider naevi) on the patient AR4's chest and back before and after one and two years of AHCC

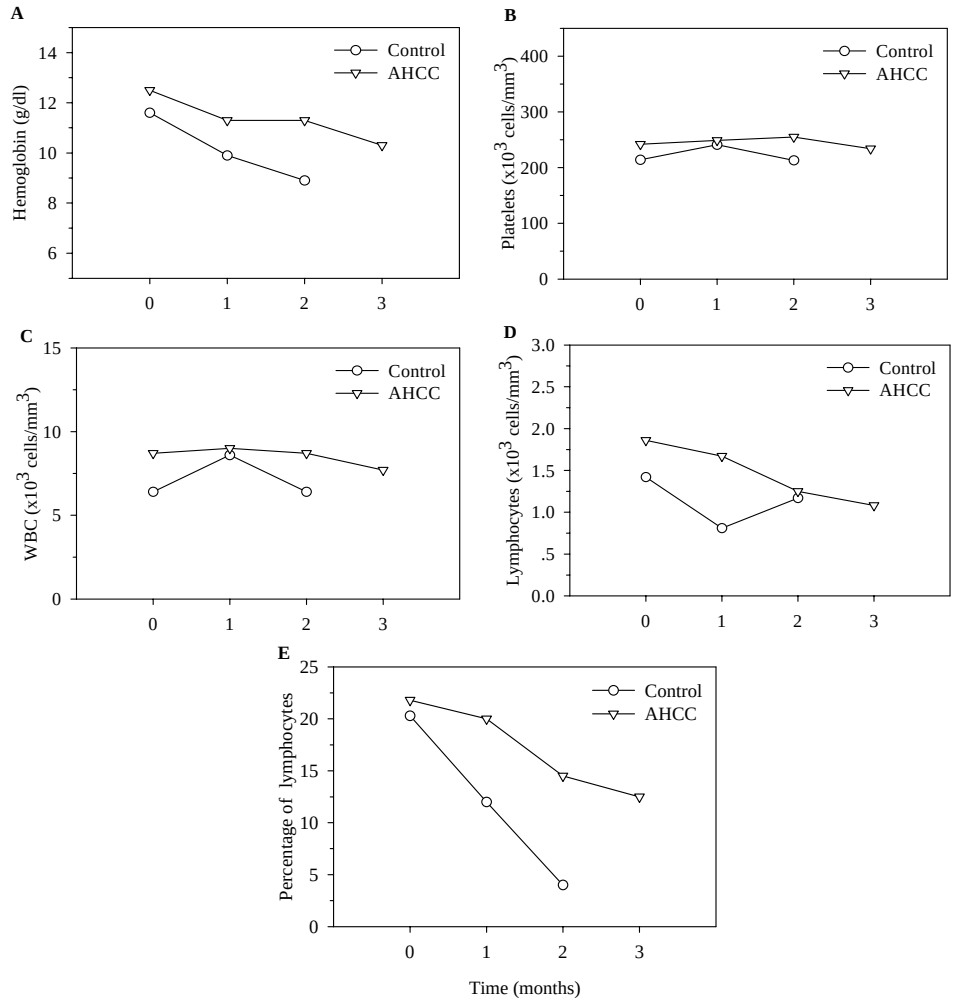

Fig. 3 The median profiles of hematological parameters of patients. The open circles indicate the levels of the appropriate parameters in the control group, and the open triangles represent the levels in the AHCC treated-group. The medians of these parameters are: (A) hemoglobin, (B) platelet counts, (C) white blood cell counts, (D) total lymphocytes, and (E) lymphocytes (%).

treatment. The spider naevi disappeared after three months of treatment with no new occurrence until two years of follow-up. Pictures of his liver mass using magnetic resonance imaging from the year of 2002 (the start of treatment) to 2005 showed that there was no change in tumor size and no new lesion appeared (Fig. 7). These images were also consistent with his good prognosis and longer survival time.

DISCUSSION

The natural course of the hepatocellular carcinoma is by rapid progression with a median survival of 4.1 months.[25,26] Early detection of liver cancer has recently become possible because of the progress in diagnostic imaging. This led to an increased incidence of resection of early-stage liver cancer during the last decade.[10] Although hepatic resection is the most effective treatment of liver cancer, most patients are not eligible as they still are diagnosed at a late stage.[7,8] To prolong survival in these patients, the most widely used options are adjuvant chemotherapies,[27] even though there is no clear evidence that their administration results in improved survival.[28] Thus, the search for other potentially useful therapeutic approaches is still necessary. Recently, there have been many attempts to treat the cancer by

Table 3 Biochemical parameter results of patients from AHCC treated- and control groups

	AHCC	Control	*p*-value
Albumin (g/dl)			
M0	(N = 33)	(N = 10)	
Median (IQR)	3.7 (3.4-4.9)	3.5 (2.9-3.8)	0.194
M1	(N = 32)	(N = 7)	
Median (IQR)	3.4 (3.0-4.0)	2.9 (2.4-2.9)	0.000
M2	(N = 25)	(N = 2)	
Median (IQR)	3.5 (3.0-4.0)	2.6	0.023
Total bilirubin (mg/dl)			
M0	(N = 33)	(N = 10)	
Median (IQR)	0.8 (0.6-2.0)	2.7 (1.0-6.7)	0.027
M1	(N = 32)	(N = 7)	
Median (IQR)	1.1 (0.8-1.7)	7.3 (2.3-15.3)	0.003
M2	(N = 26)	(N = 2)	
Median (IQR)	1.4 (0.7-3.1)	10.6	0.153
AST (U/l)			
M0	(N = 34)	(N = 10)	
Median (IQR)	79 (48-173)	108 (57-154)	0.516
M1	(N = 33)	(N = 7)	
Median (IQR)	81 (48-166)	177 (72-256)	0.180
M2	(N = 25)	(N = 2)	
Median (IQR)	87 (70-180)	432	0.063
ALT (U/l)			
M0	(N = 34)	(N = 10)	
Median (IQR)	51 (30-117)	39 (29-53)	0.384
M1	(N = 33)	(N = 7)	
Median (IQR)	60 (32-102)	50 (28-63)	0.344
M2	(N = 26)	(N = 2)	
Median (IQR)	64 (36-116)	123	0.222
Alkaline phosphatase (U/l)			
M0	(N = 34)	(N = 10)	
Median (IQR)	194 (126-340)	223 (167-303)	0.327
M1	(N = 33)	(N = 10)	
Median (IQR)	200 (114-325)	250 (150-407)	0.328
M2	(N = 26)	(N = 2)	
Median (IQR)	198 (111-424)	196	0.592

M, month; N, number of patients; IQR, interquartile range

stimulating the patient's immune system. Several biological response modifiers (BRMs) have been developed such as BCG, Picibanil, PS-K, interferon, and interleukin-12;[29-31] however, the clinical efficacy of these substances remains uncertain.

The interest in the administration of functional food such as AHCC as a BRM has been elucidated in many studies. *In vitro* experiments have shown that AHCC restored NK cell activity that was depressed by an anti-cancer drug, and also stimulated peritoneal macrophage cytotoxicity, NO production, and cytokine production. Both NK cells and macrophages have been found to be involved in the inhibition of tumor metastasis following activation by BRMs.[17] AHCC induced enhancement of the immune response in cancer patients *via* enhancement of IL-12 and IFNγ production, and NK activity.[24] Therefore, the AHCC effect may be mediated by the natural host immunity which is restored or activated by AHCC. These findings suggest that AHCC may induce its therapeutic effect on the survival of the

Table 4 Hematological parameter results of patients from AHCC treated- and control groups

	AHCC	Control	*p*-value
Hemoglobin (g/dl)			
M0	(N = 34)	(N = 10)	
Median (IQR)	12.5 (11.0-13.4)	11.6(10.1-13.4)	0.385
M1	(N = 32)	(N = 7)	
Median (IQR)	11.3 (10.1-13.9)	9.9 (9.0-11.5)	0.088
M2	(N = 26)	(N = 2)	
Median (IQR)	11.3 (10-13.1)	8.9	0.090
WBC ($x10^3$/μl)			
M0	(N = 34)	(N = 10)	
Median (IQR)	8.7 (6.4-10.8)	6.4 (4.8-9.3)	0.145
M1	(N = 32)	(N = 10)	
Median (IQR)	9.0 (6.3-10.9)	8.6 (6.0-14.3)	0.783
M2	(N = 26)	(N = 2)	
Median (IQR)	8.7 (7.3-9.7)	6.4	0.532
Platelet ($x10^3$/μl)			
M0	(N = 34)	(N = 10)	
Median (IQR)	242 (208-340)	214 (152-318)	0.449
M1	(N = 32)	(N = 7)	
Median (IQR)	249 (181-365)	241 (168-300)	0.770
M2	(N = 26)	(N = 2)	
Median (IQR)	255 (203-317)	213	0.562
Total lymphocyte ($x10^3$/μl)			
M0	(N = 34)	(N = 10)	0.101
Median (IQR)	1.9 (1.4-2.3)	1.4 (0.8-1.9)	
M1	(N = 32)	(N = 7)	
Median (IQR)	1.7 (1.2-2.1)	0.8 (0.7-2.1)	0.323
M2	(N = 26)	(N = 2)	
Median (IQR)	1.3 (0.8-2.2)	1.2	0.532
Lymphocyte (%)			
M0	(N = 34)	(N = 10)	
Median (IQR)	21.8 (16.8-30.0)	20.3 (16.7-24.4)	0.401
M1	(N = 32)	(N = 7)	
Median (IQR)	20.0 (12.4-25.8)	12.0 (6.0-22.0)	0.103
M2	(N = 26)	(N = 2)	
Median (IQR)	14.5 (10.2-25.3)	4.0	0.026

M, month; N, number of patients; IQR, interquartile range

cancer patients as a result of NK cell and macrophage activation mediated through host immunomodulation. Recently, AHCC has been reported to improve prognosis of postoperative hepatocellular patients with significantly longer remission periods, an increased overall survival rate and improvement of liver function.[10]

In this study, the patients with advanced liver cancer were randomized into AHCC treated- and placebo (control) groups. The preliminary results from 23 patients (13 patients in the AHCC treated- and 10 patients in the control groups) showed that all 10 (100%) patients in the control group died within 3.5 months whereas 8 (61.5%) patients in the AHCC group were still alive after that period. The significant difference in overall survival was determined by comparing median survival time of 4 and 1.5 months in the AHCC treated- and the control groups, respectively (p = 0.002, Mann-Whitney test). From this

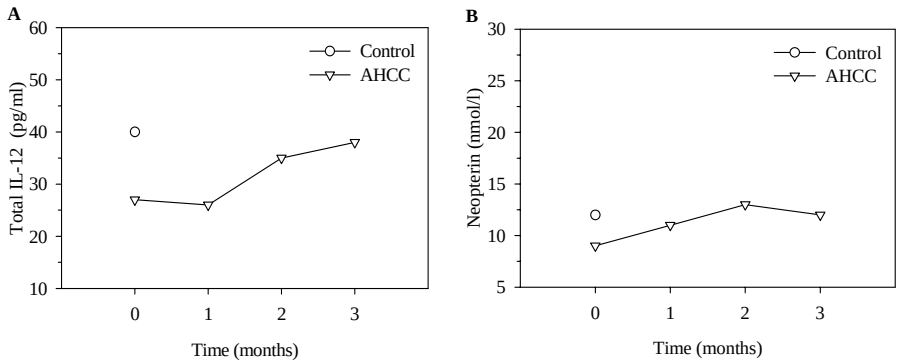

Fig. 4 The median profiles of immunological parameters of patients. The open circles indicate the levels of the appropriate parameters in the control group, and the open triangles represent the levels in the AHCC treated-group. The medians of these parameters are: (A) total interleukin-12 (IL-12), and (B) neopterin.

Table 5 Immunological parameter results of patients from AHCC treated- and control groups

	AHCC	Control	*p*-value
Interleukin-12 (pg/ml)			
M0	(N = 24)	(N = 6)	
Median (IQR)	27 (9-81)	40 (24-76)	0.432
M1	(N = 16)	(N = 1)	
Median (IQR)	26 (14-78)	46	NA
M2	(N = 11)		
Median (IQR)	35 (19-102)		NA
Neopterin (nmol/l)			
M0	(N = 29)	(N = 7)	
Median (IQR)	9 (5-14)	12 (8-31)	0.270
M1	(N = 16)	(N = 1)	
Median (IQR)	11 (8-16)	24	NA
M2	(N = 12)		
Median (IQR)	13 (8-18)		NA

M, month; N, number of patients; IQR, interquartile range; NA, no analysis

interim analysis, it seemed unethical to randomize more patients into the control group; so after this all newly enrolled patients were treated with AHCC.

Our results from 44 patients (34 AHCC treated and 10 controls) suggested that the use of AHCC increased the probability of prolonged survival and of a higher quality of life. Furthermore, it was observed that the follow-up levels of albumin, AST and ALT activity, and percentage of lymphocytes, did not deteriorate as rapidly after AHCC treatment as in the controls. This suggested that AHCC intake had some beneficial effects on maintaining the physiological status of these patients until the last 2 months of their life. The parameters that showed an apparent difference between the two groups within 1-2 months were albumin and percentage of lymphocytes. Thus, a follow-up of these two parameters might indicate an early prognosis during treatment in terms of better or worse outcome.

The immunological parameters consisted of Th-1 cytokines: IFNγ and IL-12 representing cell-mediated immunity *via* NK and macrophage activity. In addition, neopterin, a marker for macrophage activation, was determined. Elevated levels of neopterin have been reported causing a stimulation of cellular immunity including malignancies,[32,33] and are

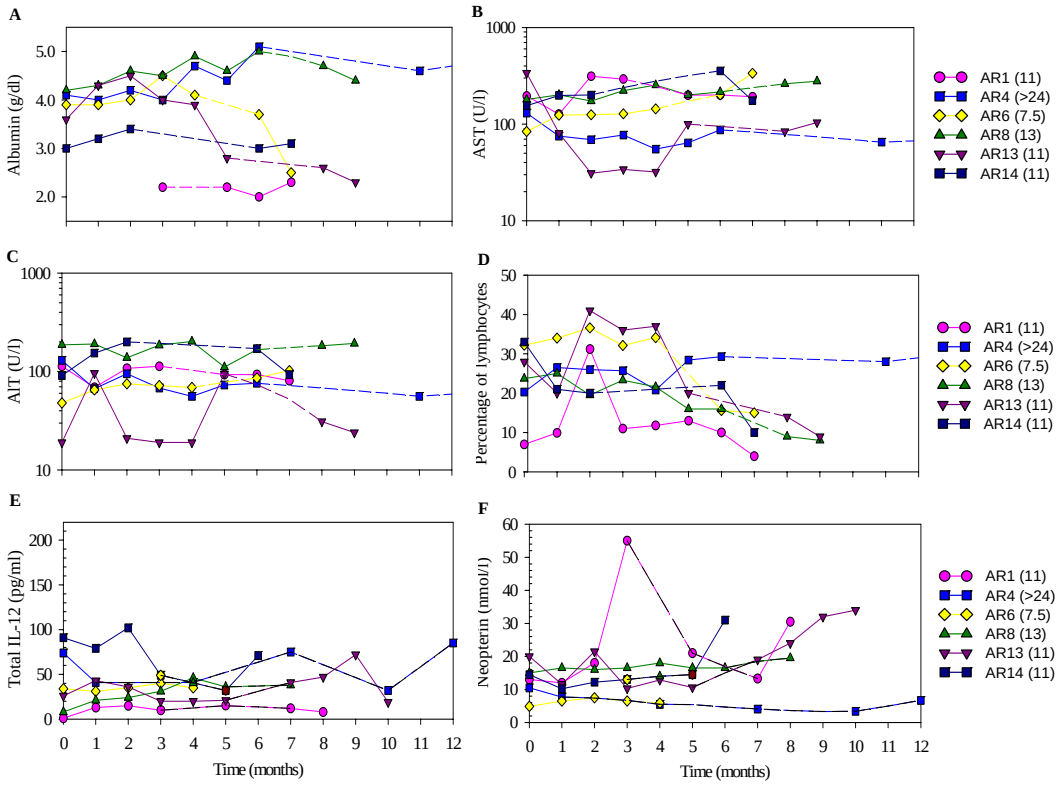

Fig. 5 The follow-up of clinical parameters in six AHCC-treated patients having longer survival (> 7 months): AR1, AR4, AR6, AR8, AR13 and AR14. The numbers in parentheses indicate patients' survival time in months. The parameters are: (A) albumin, (B) aspartate aminotransferase (AST), (C) alanine aminotransferase (ALT), (D) percentage of lymphocytes, (E) total IL-12 (IL-12), and (F) neopterin. Each dashed line was drawn to join the discontinuous data from particular months during follow-up.

also an indicator for conditions related to impaired cellular immunity. In our study, the plasma levels of IFNγ both in the AHCC treated- and control patients were lower than the detection limit of the ELISA assay. This might be because IFNγ acts locally and has a short half-life. Thus it might be necessary to measure at mRNA expression level by real-time RT-PCR because of its higher sensitivity.[34] This method might reveal possible differences between the two groups if there were any. During follow-up the plasma levels of total IL-12 in AHCC treated-patients showed a slight increase, but remained within the expected value, and the levels of neopterin were fairly stable responding to AHCC until the last 1-2 months of the patient's life. Although our results could not prove that AHCC resulted in an increase of IL-12 and stable neopterin levels because we could not compare to the controls, they showed a tendency which was consistent with the patients' prognosis and survival.

Our study suggests that AHCC helps to prolong the survival of patients with advanced liver cancer. AHCC might improve the prognosis and also delay the gradual decline of the physiological status especially of patients with longer survival compared to the controls. However, the effects of AHCC and the mechanisms responsible for its effects on the survival of patients with advanced liver cancer were not clearly explained and required more detailed studies.

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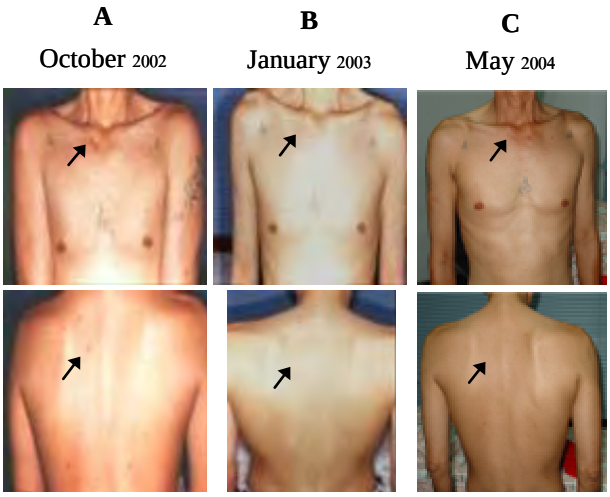

Fig. 6 Photographs of spider naevi on patient (AR4)'s chest (upper) and back (lower). Arrow indicates the spider naevi from (A) October 2002; (B) January 2003; and (C) May 2004. During period of follow–up, the spider naevi disappeared and no new lesion occurred.

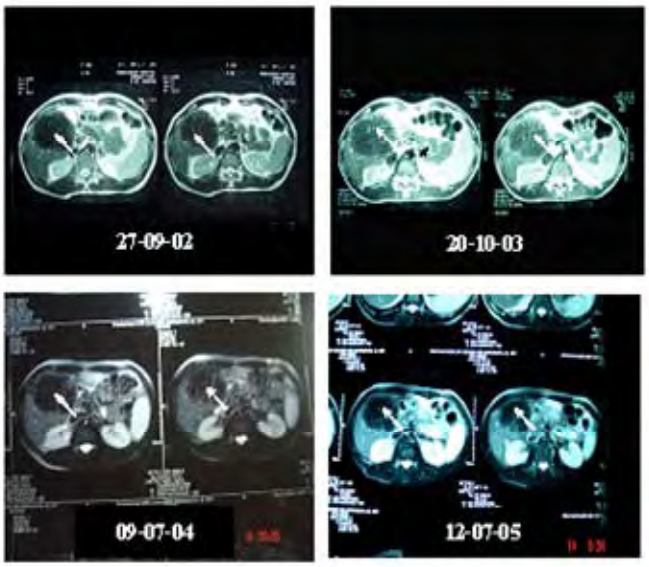

Fig. 7 Magnetic resonance imaging of liver mass of a patient (AR4) during September 2002 to July 2005. Arrow indicates lesion of the tumor. During period of follow-up, there was no change in size of tumor and no occurring of new lesion.

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