HCC, including tumor stage progression and portal vein invasion, and in predicting patient outcome. Further studies will be necessary to establish the value of the NX-DCP ratio as a tumor marker for HCC in patients taking warfarin. In addition, the value of the NX-DCP ratio was evaluated only in patients who were taking the vitamin K antagonist warfarin: its value was not evaluated in HCC patients in whom vitamin K is reduced or absent through other mechanisms such as heavy alcohol intake or nutritional deficiency. The value of the NX-DCP ratio as a marker for HCC should be confirmed for these subpopulations in the future.

In conclusion, the novel NX-DCP ratio identified elevation of DCP due to HCC in patients taking the vitamin K antagonist warfarin. Thus, by using this ratio, DCP can be used as a marker for HCC even in patients taking warfarin.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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Impact of pretreatment serum cholinesterase level in unresectable advanced hepatocellular carcinoma patients treated with sorafenib

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Abstract. The value of serum cholinesterase (ChE) level as a predictive marker in sorafenib therapy for advanced hepatocellular carcinoma (HCC) has not yet been investigated. The present retrospective study therefore analyzed the impact of the serum ChE level in 93 patients with advanced HCC treated with sorafenib. Patients were categorized into two groups: group A with pretreatment serum ChE ≥140 IU/l (n=46) and group B with pretreatment serum ChE <140 IU/l (n=47). The correlation between clinicopathological findings, including serum ChE level, and overall survival (OS) and liver damage during sorafenib therapy was investigated. The median OS of the patients was 275 days, while OS was markedly higher in group A compared to group B (P=0.002). In 70 Child-Pugh A patients, serum ChE level was a significant prognostic predictor in multivariate analysis [P=0.019, hazard ratio (HR) =2.612; 95% confidence interval (CI), 1.174-5.810]. During sorafenib treatment, 22 patients developed liver dysfunction of grade 3 or higher. Only two group A patients (4.3%) developed liver dysfunction, compared to 20 group B patients (42.6%) (P<0.001). Multivariate analysis demonstrated that the pretreatment serum ChE level was the strongest predictor of liver damage (P=0.002, HR=0.061, 95% CI: 0.010-0.373), indicating serum ChE <140 IU/l to be the only independent predictor associated with severe liver function damage during sorafenib treatment in 70 patients with grade A Child-Pugh (P=0.016; HR=0.122; 95% CI, 0.022-0.676). In conclusion, lower serum ChE level is a significant predictor of poor prognosis and severe liver damage in HCC patients treated with sorafenib. Advanced HCC patients with lower serum ChE levels, including those with a Child-Pugh A pretreatment liver function score, should be given sorafenib therapy with caution.

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Key words: cholinesterase, sorafenib, hepatocellular carcinoma, prognostic factor, liver dysfunction

Introduction

Hepatocellular carcinoma (HCC) is generally considered to be chemoresistant, and the results of systemic chemotherapy have been unsatisfactory (1). Sorafenib (Nexavar, Bayer Healthcare Pharmaceuticals, Pittsburgh, PA, USA) is a multi-kinase inhibitor that blocks tumor growth and cell proliferation, and was the first systemic chemotherapeutic agent found to improve the survival time of patients with advanced HCC, in the SHARP as well as Asia-Pacific trials (2-5). However, findings of these trials showed that several patients with advanced HCC remained refractory to sorafenib, and the factors determining the patients benefiting from sorafenib therapy remain unclear (2-5).

Although several studies have investigated the prognostic factors in sorafenib treatment, no consensus factors have yet been identified (6-12). Pretreatment liver function parameters, such as Child-Pugh classification, serum bilirubin, serum albumin (ALB) and serum aminotransferase have been reported as prognostic factors in sorafenib treatment (6-12).

Serum cholinesterase (ChE) in combination with ALB is one of the main indices of the protein-synthetic ability of the liver (13-19). Unlike ALB level, which is influenced by various factors such as bleeding, inflammation, chronic renal diseases or branched chain amino acid administration, the serum ChE level simply reflects the background liver function (13-19). Serum ChE level has been reported to be an important prognostic factor in malignancies other than HCC, including gastric and pancreatic cancers treated with systemic chemotherapy (13-17). In addition, serum ChE level has been reported as a prognostic factor in cholangiocellular carcinoma patients treated with radiotherapy (18), patients with recurrent HCC after hepatectomy (20), and in liver transplantation recipients with chronic end-stage liver disease (21). Serum ChE thus seems to be significantly involved in the treatment of several malignancies (22,23). However, to the best of our knowledge, no studies have yet investigated the value of serum ChE level as a predictive marker in sorafenib

therapy for advanced HCC. In the present study, we therefore focused on serum ChE level as an index of liver function and investigated its significance in advanced HCC patients treated with sorafenib.

Patients and methods

Patients. A total of 102 patients with unresectable HCC were treated with sorafenib at the Department of Gastroenterology and Hepatology (Osaka Red Cross Hospital, Osaka, Japan) between June, 2009 and February 2012. The indications for sorafenib therapy were: unresectable advanced HCC determined by dynamic computed tomography (CT) scan; Eastern Cooperative Oncology Group (ECOG) performance status of 0-2; pretreatment Child-Pugh classification of A or B; presence of extrahepatic metastases; refractory to previous HCC therapies, such as transcatheter arterial chemoembolization (TACE); unsuitability for TACE for anatomical reasons and absence of uncontrollable ascites. Pretreatment serum ChE levels were not measured in nine (8.8%) of the 102 patients, and the present study population therefore consisted of 93 patients with measured pretreatment serum ChE levels.

Study protocol. The median pretreatment serum ChE level was 138 IU/l (range, 31-276 IU/l). Patients were therefore categorized into two groups: group A with a pretreatment serum ChE level ≥140 IU/l (n=46) and group B with a pretreatment serum ChE level <140 IU/l (n=47). We retrospectively analyzed the correlation between overall survival (OS), and pretreatment serum ChE level as well as other pretreatment clinicopathological variables including age, gender, cause of liver disease, Child-Pugh classification, pretreatment tumor characteristics such as tumor node metastasis (TNM) stage, Barcelona Clinic Liver Cancer (BCLC) stage, portal vein tumor invasion and metastatic sites, tumor markers, laboratory data such as total bilirubin (TBIL), ALB, aspartate aminotransferase (AST), alanine aminotransferase (ALT), prothrombin time and serum creatinine, as well as the presence of ascites. We also examined the correlation between the development of severe liver damage during sorafenib therapy, and pretreatment ChE level and the above-mentioned clinicopathological variables. Severe liver damage was defined as: liver dysfunction occurring within 3 months from the administration of sorafenib, including elevated AST, ALT and TBIL, and hepatic encephalopathy or liver failure of grade 3 or higher based on the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. In addition, we performed subgroup analyses on patients with good liver function defined as Child-Pugh class A. Written informed consent was obtained from the patients prior to sorafenib therapy. This retrospective study protocol was in compliance with the provisions of the Declaration of Helsinki.

Initial sorafenib dose and treatment discontinuation. The initial sorafenib dose was determined according to factors, such as patient body weight, body surface area, age, comorbid diseases, performance status and liver function. The initial sorafenib dose in this study ranged from 400 to 800 mg/day. This took into account the fact that studies in several countries, including Japan, have reported serious adverse events in several advanced HCC patients administered an initial sorafenib dose of 800 mg/day, leading to treatment discontinuation. The initial sorafenib dose was therefore determined taking this fact into consideration. Sorafenib treatment was continued until disease progression, unacceptable drug-related toxicity or the patient's decision to discontinue.

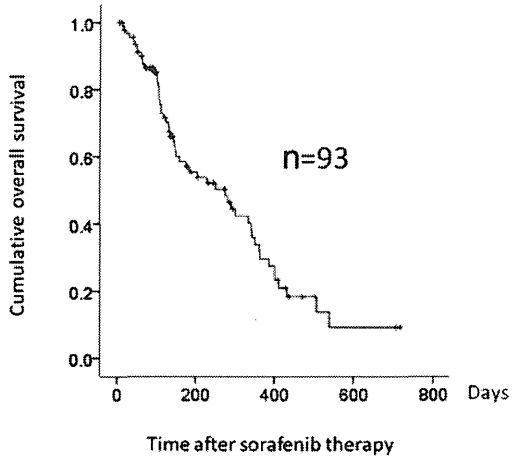

Figure 1. Cumulative overall survival (OS) for all the cases (n=93). The median OS was 275 days.

Statistical analysis. OS curves were generated using the Kaplan-Meier method and compared using log-rank tests. OS was calculated from the initial date of sorafenib treatment until death by any cause or until the last follow-up. Serum ChE level and other clinicopathological variables were analyzed using univariate and multivariate analyses. Regarding OS, the Cox proportional hazard model was used for multivariate analysis of factors considered significant in univariate analysis. Associations between pretreatment serum ChE level and additional clinicopathological variables, and the development of liver damage during sorafenib treatment were also examined using Fisher's exact tests. Regarding the development of liver damage, the variables found to be significant in the univariate analysis were subjected to the multivariate analysis using logistic regression analysis. Data were presented as the median value (range). $P<0.05$ was considered to indicate a statistically significant difference. Statistical analyses were carried out using the SPSS software (SPSS for Windows 15.0, SPSS, Inc., Chicago, IL, USA).

Results

Patient characteristics. The baseline characteristics of the two groups are shown in Table I. Group A comprised 37 males and 9 females, with a median age of 70 years (range, 46-86). Group B comprised 35 males and 12 females, with a median age of 71 years (range, 47-89). With regard to tumor characteristics, 22 patients in group A and 19 patients in group B had extrahepatic metastases, while 29 and 27 patients were classified with BCLC stage C disease in groups A and B, respectively. Most patients had received previous therapies for HCC: one or more sessions of TACE had been performed in 75 patients, radiofrequency ablation or percutaneous ethanol

Table I. Baseline characteristics of patients with advanced hepatocellular carcinoma.

Variable	Group A (n=46) N or median value (range)	Group B (n=47) N or median value (range)	P-value
Age (years)	70 (46-86)	71 (47-89)	0.779[a]
Gender (male/female)	37/9	35/12	0.794[b]
Body surface area (m^2)	1.59 (1.09-2.18)	1.58 (1.38-1.89)	0.852[a]
Etiology of liver disease			
Hepatitis B/hepatitis C/non-B non-C	10/24/12	7/32/8	0.341[b]
TNM stage			
Stage II/III/IVA/IVB	3/14/7/22	1/18/9/19	0.822[b]
Site of metastases (yes/no)			
Lung	13/33	7/40	0.136[b]
Bone	8/38	8/39	1.000[b]
Adrenal	1/45	2/45	1.000[b]
Lymph node	11/35	9/38	0.621[b]
Portal vein tumor invasion (yes/no)	8/38	10/37	0.794[b]
ECOG PS, 0/1/2	40/5/1	40/4/3	0.802[b]
Child-Pugh classification, A/B	41/5	29/18	0.003[b]
BCLC stage, B/C	17/29	20/27	0.673[b]
Pretreatment serum AFP (ng/ml)	476 (2.2-270,300)	98 (2.9-688,400)	0.251[a]
Pretreatment serum DCP (mAU/ml)	1935 (10-98,510)	937 (11-421,210)	0.462[a]
Previous therapies for HCC (yes/no)			
TACE	40/6	35/12	0.322[b]
RFA or PEIT	15/31	12/35	0.649[b]
Surgery	9/37	6/41	0.106[b]
Radiation	5/41	11/36	0.089[b]
Initial sorafenib dose (800/400 mg per day)	13/33	13/34	1.000[b]
Treatment response			
CR/PR/SD/PD/NE	0/9/9/14/14	1/2/11/10/23	0.410[b]

[a]Unpaired t-test; [b]Fisher's exact test; N, number; TNM, tumor node metastasis; ECOG PS, Eastern Cooperative Oncology Group Performance Status; BCLC, Barcelona Clinic Liver Cancer; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization; RFA, radiofrequency thermal ablation; PEIT, percutaneous ethanol injection therapy; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluated.

injection therapy in 27, hepatectomy in 15 and palliative radiation therapy in 16 patients. With regard to pretreatment liver function, 73 patients (78.5%) had Child-Pugh A and 20 (21.5%) had Child-Pugh B function. The incidence of patients with Child-Pugh A status was significantly higher in group A compared to group B (P=0.003).

Sorafenib was initiated at 800 mg/day in 26 patients (28.0%) and at 400 mg/day in 67 patients (72.0%). With regard to treatment response, complete response was obtained in 1 patient, partial response (PR) in 10 patients, stable disease in 26 and progressive disease in 25 patients, based on the modified Response Evaluation Criteria in Solid Tumor (mRECIST) (24).

Predictive factors for OS and causes of mortality. The median observation period for the analyzed cases was 136 days (range, 3-716), while the median OS was 275 days (Fig. 1). Fifty-three patients (57.0%) succumbed to the disease during the observation period. The causes of mortlity were HCC progression in 44 patients, liver failure in 3 and miscellaneous causes in 6 patients. The median OS was 319 and 106 days for group A and B patients, respectively (P=0.002) (Fig. 2).

Univariate analysis revealed the presence of bone metastases (P=0.005), ALB level ≥3.5 g/dl (P=0.042), serum ChE level ≥140 IU/l (P=0.002) and the presence of ascites (P=0.001) to be significant independent factors linked to OS (Table II). However, multivariate analyses of the four factors found to be significant by univariate analysis revealed only bone metastases (P=0.018) and the presence of ascites (P=0.011) to be significant independent factors linked to OS (Table II). Serum ChE level tended to correlate with OS, though the correlation was not significant in multivariate analysis (P=0.068) (Table II).

Table II. Univariate and multivariate analyses of factors contributing to overall survival in the cases (n=93).

		Univariate analysis	Multivariate analysis		
Variable	N	P-value[a]	HR	95% CI	P-value[b]
Age ≥70 years (yes/no)	43/50	0.960			
Gender (male/female)	76/17	0.308			
HBsAg-positive (yes/no)	17/76	0.446			
Child-Pugh classification, A/B	70/23	0.099			
TNM stage, stage III/stage IVA or IVB	37/56	0.149			
BCLC stage, B/C	37/56	0.375			
Portal vein tumor invasion (yes/no)	18/75	0.377			
Bone metastases (presence/absence)	16/77	0.005	2.45	1.168-5.172	0.018
Serum AFP ≥320 ng/ml (yes/no)	47/46	0.126			
Serum DCP ≥1000 mAU/ml (yes/no)	49/44	0.344			
Total bilirubin ≥1 IU/l (yes/no)	34/59	0.653			
Serum albumin ≥3.5 g/dl (yes/no)	37/56	0.042	1.15	0.560-2.353	0.705
AST >50 IU/l (yes/no)	51/42	0.424			
ALT >50 IU/l (yes/no)	30/63	0.671			
Serum cholinesterase ≥140 IU/l (yes/no)	46/47	0.002	0.52	0.259-1.049	0.068
Prothrombin time ≥70% (yes/no)	82/11	0.192			
Serum creatinine ≥1 IU/l (yes/no)	25/68	0.643			
Ascites (presence/absence)	20/73	0.001	2.07	1.180-3.628	0.011

[a]Log-rank test; [b]Cox proportional hazards model; N, number; HBsAg, hepatitis B surface antigen; TNM, tumor node metastasis; BCLC, Barcelona Clinic Liver Cancer; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HR, hazard ratio; CI, confidence interval.

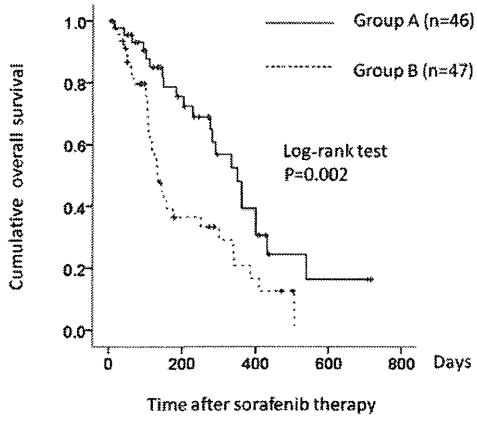

Figure 2. Cumulative overall survival (OS) according to serum cholinesterase (ChE) level. OS was significantly longer in group A patients (serum ChE ≥140 IU/l, n=46) compared to group B patients (serum ChE <140 IU/l, n=47) (P=0.002).

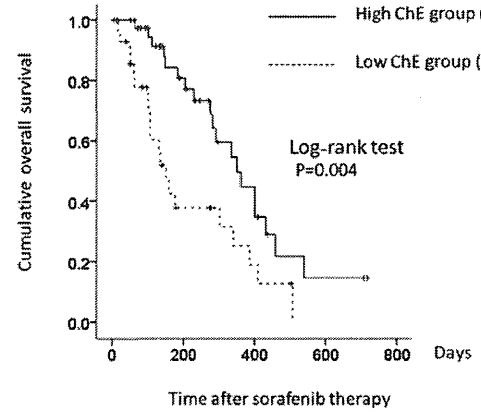

Figure 3. Cumulative overall survival (OS) according to serum cholinesterase (ChE) level in patients with Child-Pugh A (n=70). OS was markedly longer in patients with higher ChE levels (serum ChE ≥140 IU/l, n=41) compared to those with lower ChE levels (serum ChE <140 IU/l, n=29) (P=0.004).

Subgroup analyses in patients with Child-Pugh A. A statistically significant difference was detected between the two groups in terms of baseline Child-Pugh classification. We therefore performed subgroup analyses based on Child-Pugh status. We examined 70 patients with Child-Pugh class A liver function, of whom 41 (58.6%) had higher serum ChE (≥140 IU/l) and 29 (41.4%) had lower ChE (<140 IU/l). Median OS was 350 and 150 days in the higher and lower ChE groups, respectively (P=0.004) (Fig. 3). Presence of bone metastases (P=0.010) and serum ChE level (P=0.004) were markedly associated with OS in univariate analyses (Table III), and were significant independent factors linked to OS in Child-Pugh A patients, according to multivariate analyses (Table III).

Table III. Univariate and multivariate analyses of factors contributing to overall survival in patients with Child-Pugh A (n=70).

		Univariate analysis	Multivariate analysis		
Variable	n	P-value[a]	HR	95% CI	P-value[b]
Age ≥70 years (yes/no)	35/35	0.833			
Gender (male/female)	57/13	0.149			
HBsAg positive (yes/no)	14/56	0.786			
TNM stage, stage III/stage IVA or IVB	28/42	0.132			
BCLC stage, B/C	28/42	0.163			
Portal vein tumor invasion (yes/no)	12/58	0.175			
Bone metastases (presence/absence)	11/59	0.010	3.367	0.121-0.730	0.008
Serum AFP ≥320 ng/ml (yes/no)	34/36	0.283			
Serum DCP ≥1000 mAU/ml (yes/no)	35/35	0.526			
Total bilirubin ≥1 IU/l (yes/no)	22/48	0.843			
Serum albumin ≥3.5 g/dl (yes/no)	34/36	0.087			
AST ≥50 IU/l (yes/no)	36/34	0.324			
ALT ≥50 IU/l (yes/no)	22/48	0.786			
Serum cholinesterase ≥140 IU/l (yes/no)	41/29	0.004	2.612	1.174-5.810	0.019
Prothrombin time ≥70% (yes/no)	42/28	0.831			
Serum creatinine ≥1 IU/l (yes/no)	16/54	0.702			

[a]Log-rank test; [b]Cox proportional hazards model; N, number; HBsAg, hepatitis B surface antigen; TNM, tumor node metastasis; BCLC, Barcelona Clinic Liver Cancer; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HR, hazard ratio; CI, confidence interval.

Predictive factors for severe liver damage. Twenty-two patients (23.7%) developed grade 3 or higher liver dysfunction during sorafenib treatment, based on the CTCAE version 4.0, resulting in the interruption or discontinuation of sorafenib therapy. Most instances of severe liver dysfunction occurred within 1 month from the initiation of sorafenib treatment. Only two group A patients (4.3%) developed liver dysfunction, compared to 20 group B patients (42.6%).

Results of the univariate analysis showed that TBIL ≥1 IU/l (P=0.041), ALB <3.5 g/dl (P=0.015), AST >50 IU/l (P=0.045), ALT ≥50 IU/l (P=0.040), serum ChE level <140 IU/l (P<0.001) and the presence of ascites (P=0.003) were significantly associated with the development of liver dysfunction (Table IV). However, results of the multivariate analysis of these six factors found that only the presence of ascites (P=0.030) and serum ChE <140 IU/l (P=0.002) were significant independent factors associated with the development of liver dysfunction during sorafenib therapy (Table IV).

Subgroup analyses of severe liver damage in patients with Child-Pugh A. The occurrence of severe liver damage in 70 patients with good liver function (Child-Pugh A) was also examined. Forty-one patients (58.6%) had a serum ChE level ≥140 IU/l, while the other 29 patients (41.4%) had a serum ChE level <140 IU/l. Only two patients (4.9%) with higher ChE developed severe liver dysfunction, compared to 16 patients (55.2%) with lower serum ChE.

Univariate analysis results showed TBIL ≥1 IU/l (P=0.029), AST ≥50 IU/l (P=0.007), ALT ≥50 IU/l (P=0.029) and serum ChE (P<0.001) to be significantly associated with the development of severe liver dysfunction (Table V). Multivariate analysis of these four factors showed that pre-treatment serum ChE level was the only independent significant factor associated with the development of severe liver dysfunction (P=0.016; HR=0.122; 95% CI, 0.022-0.676) (Table V).

Discussion

The positive results of sorafenib therapy identified by the SHARP and Asia-Pacific trials have opened dimensions in the treatment of advanced HCC (2,3). However, in terms of tumor response rate, the efficacy of sorafenib is limited compared to TACE (1). The SHARP trial showed only 2% of PR in RECIST (25,26), while the Asia-Pacific trial showed only 3% PR. By contrast, Edeline *et al* (27) reported that 12% of HCC patients treated with sorafenib achieved PR using modified RECIST. Abbadessa *et al* (28) reported that HCC patients benefited from the long-lasting effects of sorafenib. In addition, we have previously treated a patient with advanced HCC with lung metastasis who achieved CR with sorafenib (29). These results suggest that a number of patients may achieve an objective response with sorafenib therapy (27-30). However, characteristics of the patients benefiting from, as well as features associated with sorafenib-resistance have yet to be elucidated.

Llovet *et al* (7) examined plasma biomarkers as predictors of outcome in HCC patients participating in the SHARP trial and concluded that none of the tested biomarkers predicted a response to sorafenib. Numerous studies have therefore inves-

Table IV. Univariate and multivariate analyses of factors contributing to the development of liver damage in the cases (n=93).

		Univariate analysis	Multivariate analysis		
Variable	N	P-value[a]	HR	95% CI	P-value[b]
Age ≥70 years (yes/no)	43/50	0.207			
Gender (male/female)	76/17	0.631			
HBsAg positive (yes/no)	17/76	0.174			
TNM stage, stage III/stage IVA or IVB	37/56	0.191			
BCLC stage, B/C	37/56	0.086			
Portal vein tumor invasion (yes/no)	18/75	0.331			
Bone metastases (presence/absence)	16/77	0.558			
Serum AFP ≥320 ng/ml (yes/no)	47/46	0.122			
Serum DCP ≥1000 mAU/ml (yes/no)	49/44	1.000			
Total bilirubin ≥1 IU/l (yes/no)	34/59	0.041	1.881	0.564-6.273	0.304
Serum albumin ≥3.5 g/dl (yes/no)	37/56	0.015	0.729	0.138-3.840	0.709
AST ≥50 IU/l (yes/no)	51/42	0.045	0.824	0.179-3.798	0.804
ALT ≥50 IU/l (yes/no)	30/63	0.04	4.269	0.933-19.545	0.061
Serum cholinesterase ≥140 IU/l	46/47	<0.001	0.061	0.010-0.373	0.002
Prothrombin time ≥70% (yes/no)	82/11	0.509			
Serum creatinine ≥1 IU/l (yes/no)	25/68	0.366			
Ascites (presence/absence)	20/73	0.003	4.154	1.146-15.055	0.030

[a]Fisher's exact test; [b]logistic regression analysis; N, number; HBsAg, hepatitis B surface antigen; TNM, tumor node metastasis; BCLC, Barcelona Clinic Liver Cancer; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HR, hazard ratio; CI, confidence interval.

Table V. Univariate and multivariate analyses of factors contributing to liver damage in patients with Child-Pugh A (n=70).

		Univariate analysis	Multivariate analysis		
Variable	N	P-value[a]	HR	95% CI	P-value[b]
Age ≥70 years (yes/no)	35/35	0.500			
Gender (male/female)	57/13	0.699			
HBsAg positive (yes/no)	14/56	0.143			
TNM stage, stage III/stage IVA or IVB	28/42	0.533			
BCLC stage, B/C	28/42	0.533			
Portal vein tumor invasion (yes/no)	12/58	0.605			
Bone metastases (presence/absence)	11/59	0.533			
Serum AFP ≥320 ng/ml (yes/no)	34/36	0.077			
Serum DCP ≥1000 mAU/ml (yes/no)	35/35	0.752			
Total bilirubin ≥1 IU/l (yes/no)	22/48	0.029	1.496	0.293-7.635	0.628
Serum albumin ≥3.5 g/dl (yes/no)	34/36	0.190			
AST ≥50 IU/l (yes/no)	36/34	0.007	2.330	0.268-20.247	0.443
ALT ≥50 IU/l (yes/no)	22/48	0.029	2.104	0.374-11.851	0.399
Serum cholinesterase ≥140 IU/l	41/29	<0.001	0.122	0.022-0.676	0.016
Prothrombin time ≥70% (yes/no)	42/28	0.708			
Serum creatinine ≥1 IU/l (yes/no)	16/54	0.516			

[a]Fisher's exact test; [b]logistic regression analysis; N, number; HBsAg, hepatitis B surface antigen; TNM, tumor node metastasis; BCLC, Barcelona Clinic Liver Cancer; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HR, hazard ratio; CI, confidence interval.

tigated biomarkers likely to predict prognosis in HCC patients treated with sorafenib, although no consensus regarding prognostic factors has been reached. Tsukui *et al* (11) reported that the Child-Pugh classification was a significant prognostic factor, while Morimoto *et al* (12) reported that the Glasgow prognostic score, Japan integrated staging score and performance status were independently associated with survival. Regarding the clinical course, skin toxicity and reduction of serum α-fetoprotein during sorafenib therapy were reported to be associated with OS and time to progression (31,32). However, to the best of our knowledge, no reports have investigated the prognostic value of the serum ChE level.

The presence of ascites and bone metastases, and serum albumin <3.5 g/dl and serum ChE level <140 IU/l were found to be significant indicators of poor OS in the univariate analysis. TNM and BCLC stage did not contribute to OS. These findings are partially in agreement with those of Tsukui *et al* (11), who reported that background liver disease-derived factors, rather than tumor-derived factors, were correlated with prognosis in advanced HCC patients treated with sorafenib. By contrast, results of the multivariate analysis showed that the presence of bone metastases was a significant adverse prognostic factor in the analyzed patients as well as in Child-Pugh A patients. Advanced HCC patients with bone metastasis frequently present with severe pain and other symptoms associated with poor quality of life (33), thus these factors are likely to be associated with poor prognosis.

In general, patients with pretreatment liver function damage, such as Child-Pugh B, elevated AST or ALT, hypoalbuminemia and the presence of ascites are considered to be intolerant to sorafenib therapy (1-5). Results of the multivariate analysis demonstrated serum ChE level to be the strongest predictive factor for severe liver dysfunction. Under conditions of poor hepatic functional reserve, reflected by lower serum ChE levels, hepatocytes may easily be damaged by systemic chemotherapy, leading to severe liver damage. Our findings suggest that the serum ChE level, as well as the above-mentioned factors, are important predictors correlated with the development of severe liver dysfunction. Consequently, clinicians should take serum ChE levels into consideration prior to initiation of sorafenib therapy.

The present study found that pretreatment serum ChE <140 IU/l was a significant poor prognostic factor in the multivariate analysis in the subgroup of patients with good liver function of Child-Pugh class A. Moreover, patients with pretreatment serum ChE <140 IU/l were more likely to develop severe liver dysfunction during sorafenib treatment compared to those with pretreatment serum ChE >140 IU/l. These findings suggest that even patients with good pretreatment liver function of Child-Pugh class A should be treated with sorafenib with caution if they have low serum ChE levels, and a favorable clinical outcome in these patients is less likely as a result of the high frequency of discontinuation of sorafenib therapy.

There were several limitations to the present study. First, this was a retrospective, single-center study. Second, the number of patients analyzed was relatively small. Third, the initial sorafenib dose varied in individual patients, which could have led to bias. Fourth, nine patients whose pretreatment ChE levels were not tested were excluded from the study, also potentially leading to bias. A larger prospective study is therefore needed to clarify the prognostic factors in advanced HCC patients treated with sorafenib. Nevertheless, the findings of this study have demonstrated that lower pretreatment serum ChE levels were a significant risk factor for poor prognosis and liver dysfunction, even in patients with pretreatment Child-Pugh A liver function, suggesting that patients with lower serum ChE levels should be treated with caution.

In conclusion, serum ChE level may be a reliable prognostic marker for the treatment of patients with advanced HCC in the sorafenib era. Clinicians should therefore be aware of serum ChE levels, as well as various other clinical findings, prior to initiation of sorafenib therapy.

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CASE REPORT

Sorafenib-induced acute interstitial pneumonia in patients with advanced hepatocellular carcinoma: report of three cases

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Abstract Little is known about acute interstitial pneumonia (AIP) induced by sorafenib therapy in patients with advanced hepatocellular carcinoma (HCC). Here, we present three patients with advanced HCC who developed AIP during sorafenib therapy, with fatal complications in two cases. Case 1 was a 76-year-old man who developed dyspnea. Chest CT showed interstitial pneumonia. Sorafenib was discontinued immediately, and prednisolone was started. His pneumonia resolved. A drug-induced lymphocyte stimulation test for sorafenib was positive. Case 2 was a 75-year-old man and case 3 was a 77-year-old man, both of whom developed high-grade fever and hypoxemia during sorafenib therapy, and were diagnosed with AIP. In spite of high-dose steroid therapy, their respiratory failure worsened and both patients died. In all three cases, serum KL-6 or surfactant protein D concentrations were elevated, and blood and sputum cultures did not grow pathogens. All three patients were smokers with restrictive lung disease on preoperative respiratory function testing, but did not have respiratory symptoms before sorafenib therapy. The clinical features of these three cases suggest that male gender, older age, smoking history, and lung disease are associated with acute sorafenib-induced AIP in patients with advanced HCC.

Keywords Acute interstitial pneumonia · Sorafenib · Hepatocellular carcinoma · Adverse event · Lung injury

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Introduction

As systemic chemotherapy has had disappointing results in patients with hepatocellular carcinoma (HCC) [1–5], HCC has generally been considered to be chemoresistant. Sorafenib, a multi-kinase inhibitor that blocks tumor growth and cell proliferation, was the first systemic chemotherapeutic agent found to improve the survival time of patients with advanced HCC in the SHARP trial and Asian Pacific trials [1, 2, 6]. Sorafenib is therefore a novel treatment for patients with advanced HCC. However, it is associated with a low tumor response rate, minimal survival advantage, and high rate of adverse events [1–4].

The reported significant adverse events caused by sorafenib include diarrhea, skin rash (including hand-foot skin reactions), fatigue, liver dysfunction, and hypertension [1–4]. However, there have been few reported cases of interstitial lung disease such as acute interstitial pneumonia (AIP) [7, 8]. Safety information for sorafenib therapy in patients with renal cell carcinoma (RCC) was presented in Japan in December 2008, and reported four cases of acute respiratory failure among 2,000 patients with RCC who had been treated with sorafenib [9]. The clinical features, risk factors, and prognostic factors of sorafenib-induced AIP are not well known at present [7, 8]. As AIP is a life-threatening condition, a better understanding of sorafenib-induced AIP is required. We report three cases of AIP during sorafenib therapy in patients with advanced HCC.

Case reports

Case 1

Case 1 was a 76-year-old man with lymph node metastases from HCC. Serum hepatitis B virus surface antigen and

hepatitis C virus (HCV) antibody were negative. He had been given medication for diabetes and a previous myocardial infarction. He was treated with radiofrequency ablation of a single 2.5 cm diameter HCC located in segment 8 of the liver, and had a favorable tumor response. Ten months later, dynamic computed tomography (CT) detected a solitary 2-cm lymph node metastasis at the hepatic hilum. He was asymptomatic with a performance status of 0. Physical examination showed no abnormalities. Laboratory testing showed a decreased serum albumin level of 3.1 g/dL (normal range, >3.7 g/dL), decreased cholinesterase concentration of 197 IU/L (normal range, >220 IU/L), and normal aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. Liver function was Child–Pugh class A. Serum α-fetoprotein (AFP) level was 2.3 ng/mL (normal range, <10 ng/mL) and serum des-γ-carboxy prothrombin (DCP) level was 2089 mAU/mL (normal range, <39 mAU/mL). Chest X-rays and CT were normal (Fig. 1). Respiratory function testing showed percent predicted vital capacity of 47 %, and he was therefore ineligible for surgical resection of the solitary lymph node metastasis. We treated him with sorafenib 400 mg once daily on an inpatient basis after obtaining written informed consent.

On the fifth day of sorafenib treatment, he developed progressive dyspnea and fever. Physical examination showed a normal blood pressure of 130/80 mmHg, respiratory rate of 22 breaths/min, pulse rate of 120 beats/min, and body temperature of 37.5 °C. Fine inspiratory crackles were audible over the right lower lung field. Pulse oximetry at rest on room air showed oxygen saturation of 93 %. Arterial blood gas analysis on room air showed a PaO2 of 64 mmHg, PaCO2 of 27 mmHg, and pH of 7.43. Laboratory testing showed an increased lactate dehydrogenase (LDH) concentration of 321 IU/L (normal range, <260 IU/L) and increased C-reactive protein (CRP) concentration of 8.6 mg/dL (normal range, <0.5 mg/dL). Liver and renal function test results were within normal limits. Chest X-rays showed diffuse ground-glass opacities (GGOs) in both lungs. Chest CT showed diffuse interstitial lung disease (Fig. 1). We suspected sorafenib-induced AIP and therefore discontinued sorafenib therapy. No pathogens were cultured from the blood, urine, or sputum. The serum KL-6 concentration was increased at 518 U/mL (normal range, <500 U/mL). A positive drug-induced lymphocyte stimulation test (DLST) for sorafenib confirmed the diagnosis of sorafenib-induced AIP. We initiated prednisolone (PSL) therapy (20 mg/day) on the day we discontinued sorafenib therapy. His symptoms and radiological findings rapidly improved over the following 5 days, and his AIP resolved completely over the following 2 weeks (Fig. 1). He was discharged 16 days after commencing PSL therapy.

Case 2

Case 2 was a 75-year-old male with a past history of pneumothorax. After hepatectomy for HCC, he developed HCV-related multinodular recurrence, and underwent four sessions of transcatheter arterial chemoembolization (TACE). After TACE, dynamic CT showed multiple nodules of recurrent HCC in his remnant liver and we

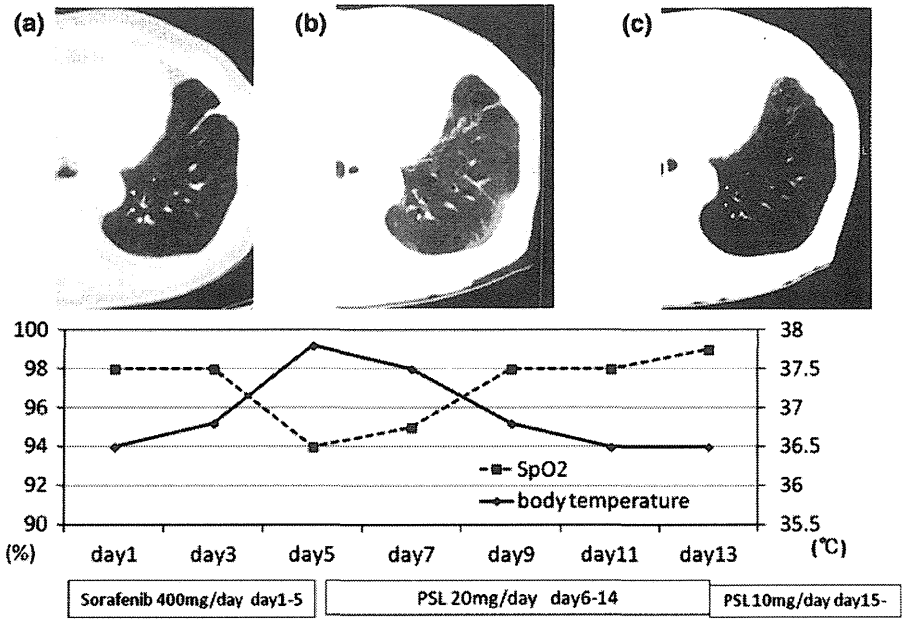

Fig. 1 Clinical course of case 1. **a** Thoracic CT scan findings before sorafenib treatment. No significant findings are present. **b** Thoracic CT scan findings on the 6th day of sorafenib treatment. Ground-glass opacities (GGOs) are present in the left lung, suggesting interstitial pneumonia. **c** Thoracic CT scan 12 days after the administration of steroid treatment showing that the GGOs have improved

administered sorafenib on an inpatient basis after obtaining written informed consent. Before administration of sorafenib, he was asymptomatic and his vital signs were within normal limits. Physical examination showed no abnormalities. He was not taking any medications. Laboratory testing showed a decreased serum albumin level of 3.4 g/dL, slightly elevated AST level of 45 IU/L, elevated AFP level of 38.5 ng/mL, and normal DCP level of 16 mAU/mL. Liver function was Child–Pugh class A. Chest X-rays and CT were normal (Fig. 2). On the second day of sorafenib therapy, he developed a low-grade fever. His symptoms progressed, and on the 11th day of sorafenib therapy he had high-grade fever and hypoxemia. His oxygen saturation at rest on room air was 92 %. We immediately discontinued sorafenib therapy. Two days later, chest X-rays showed GGOs in both lungs and chest CT showed interstitial lung disease. Laboratory testing showed increased serum concentrations of KL-6 of 1470 U/mL, surfactant protein D (SP-D) of 388 ng/mL (normal range, <109.9 ng/mL), creatine kinase of 1,006 IU/L (normal range, 40–200 IU/L), LDH of 553 IU/L, CRP of 6.6 mg/dL, and immunoglobulin E of 4,150 IU/mL (normal range, <177 IU/mL). No pathogens were cultured from blood or sputum samples.

To establish a definitive diagnosis, we performed bronchoscopy at 14 days after the onset of sorafenib therapy. The bronchial mucosa was not inflamed and we detected no pus. Bronchoalveolar lavage (BAL) showed an increased percentage of lymphocytes (43.0 %) and increased LDH concentration. No pathogens were cultured from BAL fluid samples. We diagnosed AIP, and started steroid pulse therapy with hydrocortisone (1,000 mg/day for 3 days) on the day of bronchoscopy, followed by PSL therapy (60 mg/day). His respiratory function gradually worsened (Fig. 2) and he died of respiratory failure 10 days after developing AIP.

Case 3

Case 3 was a 77-year-old man with HCV-related HCC and a huge sacral bony metastasis. He had previously been treated for hypertension and prostatic hypertrophy. He was treated with TACE for a ruptured HCC, followed by hepatectomy, a further three sessions of TACE, and palliative radiation therapy for the sacral metastasis. Although his performance status was 3 due to paralysis and pain from the bony metastasis, he wished to receive chemotherapy. Physical examination showed no abnormalities other than weakness of the legs. Blood testing showed elevated levels of AST of 58 IU/L, ALT of 63 IU/L, and ALP of 370 IU/L. Liver function was Child–Pugh class A. Chest X-rays and CT were normal (Fig. 3). We administered sorafenib 400 mg twice daily on an inpatient basis after obtaining written informed consent. He tolerated this therapy for over 4 weeks, during which time he developed grade 1 hepatic dysfunction, grade 1 hypertension, and grade 2 hand-foot skin reaction.

On the 41st day of sorafenib treatment, he developed general fatigue and high-grade fever, and we discontinued sorafenib. Two days later, he developed acute dyspnea. Chest X-rays showed diffuse GGOs in both lungs, and chest CT confirmed GGOs with septal thickening

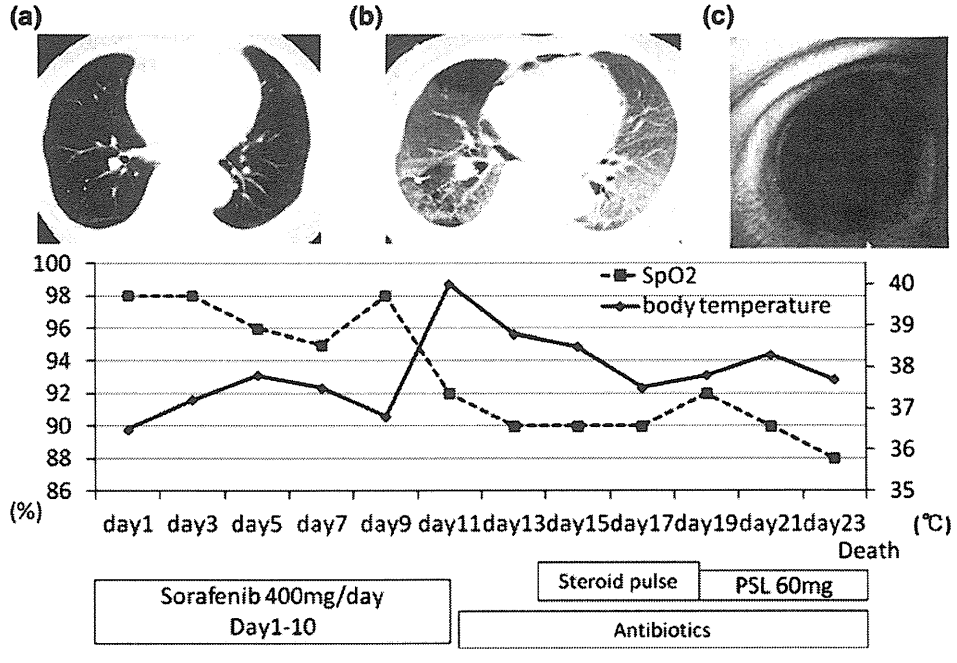

Fig. 2 Clinical course of case 2. **a** Thoracic CT scan before sorafenib treatment. No significant findings are present. **b** Thoracic CT scan findings at 13 days of sorafenib treatment. GGOs are present in both lobes, suggesting interstitial pneumonia. **c** Bronchoscopy findings at 14 days after the onset of sorafenib therapy. The bronchial mucosa was not inflamed and we detected no pus

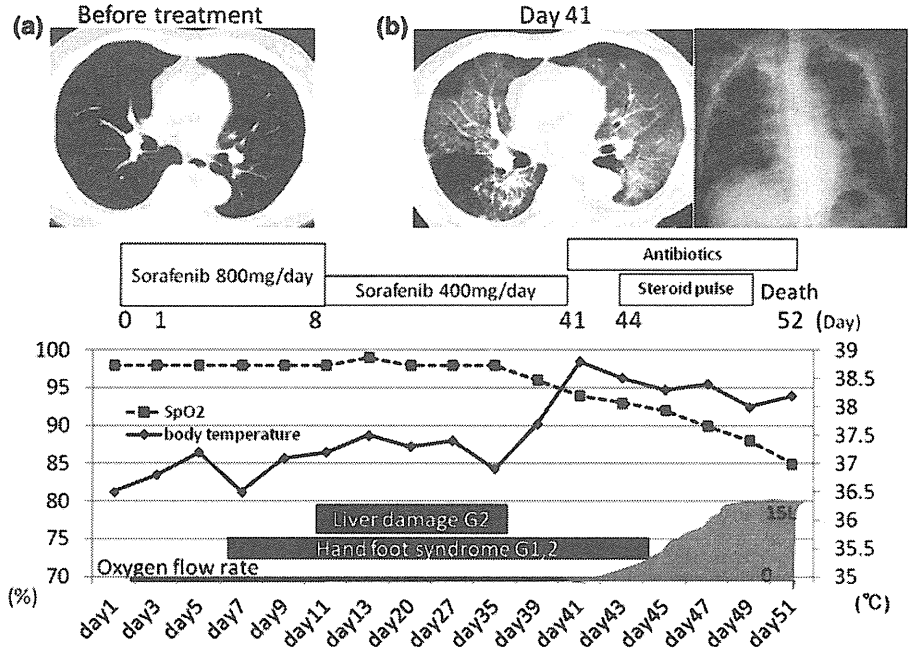

Fig. 3 Clinical course of case 3. **a** Thoracic CT scan before sorafenib treatment. No significant findings are present. **b** Thoracic CT scan and chest X-ray film after 41 days of sorafenib treatment. GGOs are present in both lungs, suggesting interstitial pneumonia

throughout both lungs. Laboratory testing showed an increased SP-D concentration of 999 ng/mL. We could not rule out the possibility of a fungal infection because his serum β-D glucan concentration was increased at 56.0 pg/mL (normal range, <20.0 pg/mL). No pathogens were cultured from the blood, urine, or sputum. We suspected sorafenib-associated AIP. Three days after discontinuing sorafenib therapy, we started hydrocortisone (1000 mg/day for 3 days) followed by PSL (30 mg/day), together with antibiotics and oxygen therapy. His respiratory status worsened and he died on the 10th day after developing dyspnea.

Discussion

Commonly reported adverse events caused by sorafenib include diarrhea, skin rash (including hand-foot skin reactions), fatigue, liver dysfunction, and hypertension. However, there have been few reported cases of sorafenib-induced interstitial lung disease [1, 2, 4, 7, 8].

From June 2009 to February 2012, we treated 105 patients with advanced HCC with sorafenib. Six of these patients (5.8 %) developed pulmonary disorders during sorafenib therapy, of which three (2.9 %) were diagnosed with probable sorafenib-induced AIP. The other three patients (2.9 %) with pulmonary disorders had acute bacterial pneumonia and/or carcinomatous lymphangitis. This was a higher incidence of AIP than reported in the all-patient post-marketing surveillance in Japan [10]. The reasons for this high incidence may include the high proportions of smokers and patients with pretreatment respiratory dysfunction in our treatment group.

Patients with advanced HCC are generally immunodeficient, making them susceptible to pneumonia [5]. Carcinomatous lymphangitis should also be considered in patients with malignancy who develop respiratory disorders. In the present three cases, dyspnea was associated with increased serum KL-6 or SP-D concentrations, and the results of blood and sputum examinations suggested that major infection was unlikely. Chest CT showed typical patterns of extensive bilateral interstitial lung disease in all patients, which was distinguishable from carcinomatous lymphangitis [11]. We therefore diagnosed probable sorafenib-induced AIP, although other conditions could not be completely excluded.

Case 1 had a positive DLST, suggesting an allergic reaction to sorafenib. Early discontinuation of sorafenib and administration of PSL resulted in rapid improvement of his respiratory function, supporting allergy as the cause of his AIP. In case 2, we made a definitive diagnosis of AIP by bronchoscopy. The absence of pus in his bronchi, and increased LDH and lymphocytes in his BAL fluid with no evidence of pathogens, helped us to rule out major infections and diagnose AIP. In case 3, it was difficult to conclusively rule out an infectious cause. However, the findings in this case were strongly suggestive of sorafenib-induced AIP.

The characteristics of, and risk factors for, sorafenib-induced AIP are not well known. Our three cases were all men aged over 75 years, and were all smokers (Brinkman index approximately 1,400 in case 1, 400 in case 2, and 800

in case 3). In addition, pretreatment respiratory function testing did not show restrictive lung disease in any of the cases (percent predicted vital capacity 47 % in case 1, 76 % in case 2, and 58 % in case 3). However, pretreatment chest CT did not show evidence of interstitial lung disease, and none of the patients had respiratory symptoms.

Several studies have reported the risk factors for gefitinib-induced AIP in patients with unresectable lung cancer [12, 13]. According to the West Japan Thoracic Oncology Group report on 1,661 patients with lung cancer who were treated with gefitinib, AIP is associated with male sex (odds ratio [OR]: 3.9), smoking (OR: 4.51), and a past history of interstitial pneumonia or respiratory disease (OR: 2.83) [13]. We observed almost the same factors in our three cases.

Table 1 shows the reported cases of sorafenib-induced AIP to date. Although results of pretreatment respiratory function testing were not reported in previous cases, one previous case had a history of interstitial lung disease. The time from initiation of sorafenib therapy to onset of respiratory failure varied from 5 days to 1 year. In all cases, chest X-ray or CT showed GGOs. Our case 2 is the first case in which bronchoscopy was performed. Five of the six cases received steroid therapy.

Drug-induced pneumonia is usually diagnosed by physical examination and X-ray and CT findings. Typical early symptoms of AIP are cough, fever, and dyspnea. Fine crackles can be heard on chest auscultation. However, drug-induced lung disease cannot be definitively diagnosed by physical, laboratory, and imaging findings. Chest X-rays at presentation are likely to miss or underestimate lung disease, but chest CT may be more useful. It is recommended that steroid therapy should be started as soon as possible [14]. In case 1, we diagnosed AIP by CT on the first day of respiratory symptoms and started steroid therapy immediately, whereas in cases 2 and 3 steroid therapy was started a few days after the onset of respiratory symptoms. In the reported fatal case of sorafenib-induced AIP in a patient with RCC, steroid therapy was started 12 days after discontinuing sorafenib. These cases indicate the importance of early diagnosis and immediate initiation of steroid therapy in patients with sorafenib-induced AIP.

The mechanisms of sorafenib-induced interstitial lung disease remain unclear. In general, drug-induced lung disease may be immune-mediated or may result from direct toxicity [15]. The present cases may reflect these two mechanisms. Case 1 had a positive DLST and case 2 had a high serum level of IgE, suggesting an immune-mediated mechanism or acute allergic reaction. Case 3 may have resulted from direct toxicity.

Recently, Myung et al. [7] suggested that sorafenib-induced pulmonary toxicity might be related to its ability

Table 1 Summary of the reported cases of sorafenib-induced AIP, including our cases

Case	Year reported	Age	Sex	Past history/ Family history	Allergy history	Brinkman index	Alcohol ingestion	Virus markers	Tumor	TNM stage	Child–Pugh score	Previous cancer therapy	%VC	FEV1.0 %	Onset of AIP	Treatment	Outcome
Our case 1	2012	76	Male	DM and OMI/ DM	None	1400	Social drinker	HCV	HCC	IVA	A	TACE, RFA	47	90	5 days	mPSL 20 mg/ day	Recovered
Our case 2	2012	75	Male	Pneumothorax/ None	None	400	Social drinker	HCV	HCC	II	A	OP, TACE, RFA	76	75	11 days	Hydrocortisone 1 g/day	Died
Our case 3	2012	77	Male	HT and PH/ None	None	800	None	HCV	HCC	IVB	A	OP, TACE, RAD	58	80	41 days	Hydrocortisone 1 g/day	Died
Myung et al.	2010	74	Male	None/NS	None	100	NS	HCV	HCC	IVA	A	TACE, RFA RAD	NS	NS	24 days	mPSL 30 mg/ day	Recovered
Ide et al.	2010	55	Male	None/NS	None	"smoker"	NS	NS	RCC	IV	NS	IL2	NS	NS	1 year	Dexamethasone 4 mg/day	Died
Bayer[a]	2008	70	Male	RA and UIP/ NS	NS	NS	NS	NS	RCC	IV	NS	None	NS	NS	40 days	None	Died

DM diabetes, *OMI* old myocardial infarction, *HT* hypertension, *PH* prostatic hypertrophy, *RA* rheumatoid arthritis, *UIP* usual interstitial pneumonia, *HCC* hepatocellular carcinoma, *RCC* renal cell carcinoma, *TNM stage* TNM classification of malignant tumors by the Union of International Cancer Control [20], *TACE* transarterial chemoembolization, *OP* operation, *RFA* radiofrequency ablation, *RAD* radiation therapy, *%VC* percent predicted vital capacity, *FEV1.0 %* percent predicted forced vital capacity in 1 s, *AIP* acute interstitial pneumonia, *mPSL* methyl prednisolone, *NS* not specified

[a] Bayer: Bayer HealthCare Japan. Safety information for Nexabar™ 200 mg tablets. December 2008 [9]

to inhibit the vascular endothelial growth factor (VEGF) signaling pathway. Several studies have reported on the relationship between regulation of VEGF and AIP [16–19]. VEGF plays a role in the maintenance of structure and function of alveolar (and other) capillaries. A decrease in the amount or activity of VEGF leads to apoptosis of bronchoalveolar cells, resulting in remodeling of the pulmonary architecture and honeycomb changes in lung structure. Many studies have reported a reduction in the amount of intrapulmonary VEGF in the early stages of lung injury, and normalization of intrapulmonary VEGF after recovery in patients with acute respiratory distress syndrome [16–19].

Sorafenib treatment, which suppresses VEGF, might induce remodeling of bronchoalveolar structures resulting in AIP. Although there are several hypotheses regarding the mechanisms of sorafenib-induced AIP, the pathways remain unclear [7, 8]. Further studies of the molecular mechanisms responsible for sorafenib-induced lung injury are required, and methods of preventing and managing such injury need further investigation.

Conclusion

We report here three cases of sorafenib-induced AIP in patients with advanced HCC. If dyspnea, cough, and high-grade fever develop during sorafenib treatment, AIP should be considered and appropriate investigation and treatment should be initiated as soon as possible. Older age, male sex, smoking history, and a history of lung disease may be risk factors for sorafenib-induced AIP. Further studies on the risk factors and the molecular mechanisms responsible for sorafenib-induced lung injury are needed.

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

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Clinical effectiveness of bipolar radiofrequency ablation for small liver cancers

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Abstract
Background Radiofrequency ablation (RFA) is minimally invasive and can achieve a high rate of cure of liver cancer. This study was conducted to evaluate the efficacy and safety of a bipolar RFA device (CelonPOWER System) in the treatment of Japanese liver cancer patients.
Methods The study was a multicenter, single-group, open-label trial. The indications for RFA were based on the Japanese guidelines for the management of liver cancer. The subjects had a Child-Pugh classification of A or B, and the target tumors were defined as nodular, numbering up to 3 lesions, each of which was 3 cm or less in diameter, or solitary lesions up to 4 cm in diameter. To test for the non-inferiority of the CelonPOWER System, this system was compared with the Cool-tip RF System, which has already been approved in Japan, in terms of the complete necrosis rate (CNR).
Results The CNR obtained with the CelonPOWER System was 97.8 % (88/90 patients). The CNR obtained with the Cool-tip RF System was 86.2 % (50/58 patients), confirming the non-inferiority of the CelonPOWER System ($p < 0.001$, Fisher's exact test based on binomial distribution). Throughout the treatment and follow-up periods, there were no adverse events regarding safety that were uniquely related to the CelonPOWER System and there were no cases of device failure.
Conclusions The CelonPOWER System was confirmed to be an effective and safe RFA device. It could become extensively used as a safe next-generation RFA device, reducing the physical burden on patients.

Keywords Small hepatocellular carcinoma · Radiofrequency ablation (RFA) · Bipolar RFA · Conformite Europeenne (CE) mark · Non-inferiority to monopolar RFA

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Introduction

According to a report of the Japanese Ministry of Health, Labor and Welfare in 2010, the number of deaths due to malignancies, including hepatocellular carcinoma (HCC), which is the most common type of primary liver cancer, has tended to increase annually [1]. In the 2007 report of the Japanese Ministry of Health, Labor and Welfare, the mortality of liver cancer was the 3rd highest among malignant diseases, following gastric cancer and lung cancer [2]. HCC appears in cirrhotic liver, and cirrhotic liver often results from alcohol abuse or chronic hepatitis B virus (HBV) or HCV infection. The presence of liver cirrhosis limits HCC treatment options, because surgery and systemic chemotherapy impair residual liver function and can induce fatal liver failure. In addition,

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even if the primary tumor is completely resected, there is a very high recurrence rate in the residual liver [3, 4].

Radiofrequency ablation (RFA) is a minimally invasive method that can yield radical localized therapeutic results, and it has become a standard treatment for small liver cancers 3 cm or less in diameter [5].

Three different RFA systems have been introduced in Japan, all consisting of monopolar devices. One of the main problems with monopolar RFA devices is that the electrical current flows between the electrodes and the grounding pad that is used in these devices. The current flows in a wide area of the body, which may cause systemic symptoms, such as heat retention and perspiration. In addition, because the applicator is distant from the grounding pad, its low energy efficiency requires a long ablation time. Moreover, energy concentration can occur owing to an unanticipated current pathway between the applicator and grounding pad, posing a risk of burns at the grounding pad patch site and at non-treatment sites [3, 6–9].

A bipolar system, in contrast to the monopolar systems, features as its principal characteristic an electrical current flowing between two electrodes on a single probe. With a bipolar system, the current pathway is limited to only within the treatment area, thus eliminating the need for a grounding pad. A bipolar RFA system also overcomes such disadvantages of a monopolar system as the occurrence of heat retention and other side effects, low energy efficacy, and thermal injuries at electrode pad sites caused by an electrical current flowing in the body. The simultaneous use of multiple applicators with a bipolar system makes it possible to achieve a sufficiently large thermocoagulation volume with a single ablation procedure. That is, one ablation is usually sufficient for a wide area and this enables a short ablation time. In addition, ablation can be achieved even if the electrodes are not inserted directly into the tumor. The use of the bipolar system with multiple applicators with a wide ablation area maximizes the effectiveness of the bipolar system.

The purpose of this study was to evaluate the safety and efficacy of a bipolar RFA device, the CelonPOWER System, in order to obtain the clinical data necessary for an application for its regulatory approval in Japan. The study and protocol were designed in compliance with Japanese good clinical practice (GCP) based on the advice from the Pharmaceuticals and Medical Devices Agency (PMDA) of the Japanese regulatory authority. In designing this study, we were requested by the PMDA to compare this device with an existing RFA device (that had been already approved in Japan) and we selected the data from the 2002 to 2003 clinical study of the Cool-tip RF System as valid control data. The study of the Cool-tip RF System was also conducted to obtain marketing approval in Japan [10]. This study was sponsored by Olympus Medical Systems Corp.

Patients, materials, and methods

Device

Celon AG Medical Instruments (Teltow, Germany) developed a bipolar RFA device (CelonPOWER System) in order to overcome the disadvantages of monopolar RFA devices. Unlike a monopolar RFA system, the prime characteristic of this new device is its bipolar feature, i.e., two electrodes are located on the same needle (Fig. 1a, b), allowing electricity flow only between the electrodes at the treatment target site, eliminating both the need for a grounding pad and the danger of burns (Fig. 2a, b).

The bipolar characteristics of the CelonPOWER system ensure the return of power to the device, and the simultaneous use of multiple applicators yields an extensive ablated area in a single treatment, which can reduce treatment time and the burden on the patient. This eliminates the need for repeated reinsertion of single monopolar needles to perform overlapping ablation. Another advantage of the bipolar device is that electric current is immediately retrieved, preventing it from flowing to unintended sites. The CelonPOWER System was awarded the Conformite Europeenne (CE) mark in 2003, and since then its use has spread mainly in Europe [11–17].

The CelonPOWER System consists of a high-frequency power generator, a water pump, and computerized applicators for regulation of the current frequency. The basic

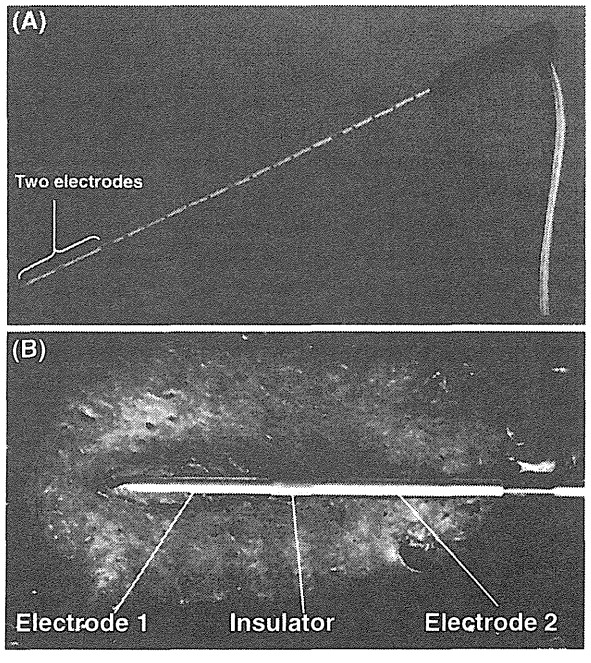

Fig. 1 In the CelonPOWER System, each applicator is needle-shaped and has two electrodes near its tip

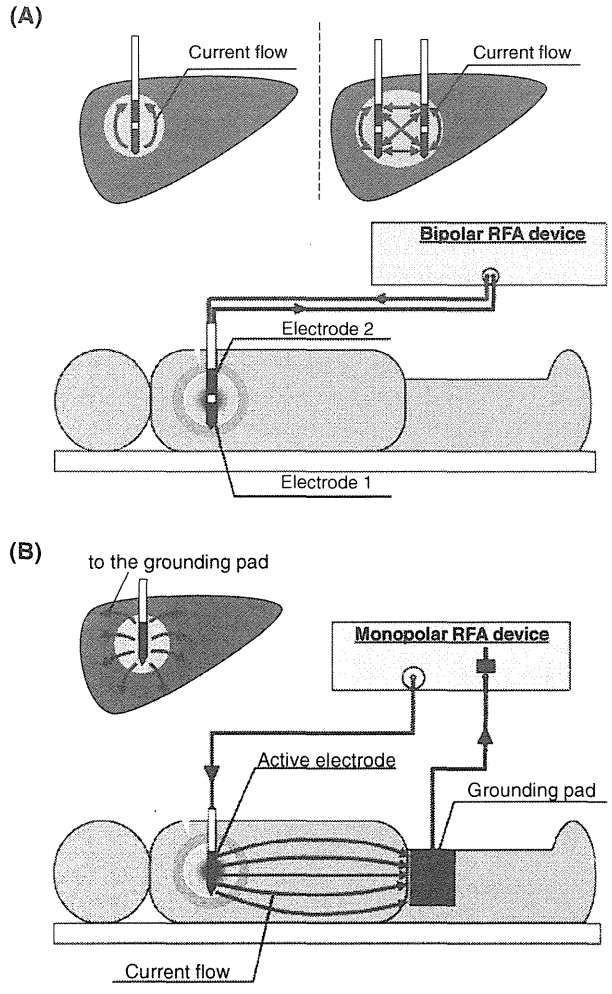

Fig. 2 Differences in the electrical flow routes of **a** the monopolar and **b** the bipolar (CelonPOWER System) radiofrequency ablation (*RFA*) systems. With the bipolar system (CelonPOWER System), the electrical current flows between the two electrodes, and for this reason the current pathway is limited to the treatment area, allowing lower power to be concentrated in a specific area and yet yielding effects equivalent to those obtained by higher energy monopolar devices, the power of which is dispersed throughout the body to the dispersion grounding pads placed under the patient

frequency of the power generator is 470 kHz, with a maximum output of 250 W. All the needles for RFA are 1.8 mm in width (15 G) but there are 3 different lengths: 20, 30, and 40 mm. The Cool-tip RF System needles are 1.5 mm in width (17 G).

Bipolar applicators

Each applicator is needle-shaped and has two electrodes near its tip. The electrical current flows between the two electrodes on the single probe, limiting the current pathway to within the treatment area. A grounding pad is unnecessary (Fig. 2a). The applicators are cooled by the internal circulation of chilled water.

Multipolar application

When simultaneously using multiple applicators (up to 3 can be employed simultaneously), it is possible to treat relatively large cancers that could not be sufficiently ablated by means of one insertion of a single applicator. The high-frequency electrical current flows sequentially between the electrodes of the applicators (6 electrode pair combinations when there are 2 applicators, 15 electrode combinations when there are 3 applicators) (Fig. 3a).

Resistance controlled automatic power (RCAP)

RCAP is a function that monitors the change of electric resistance between the electrodes, and automatically

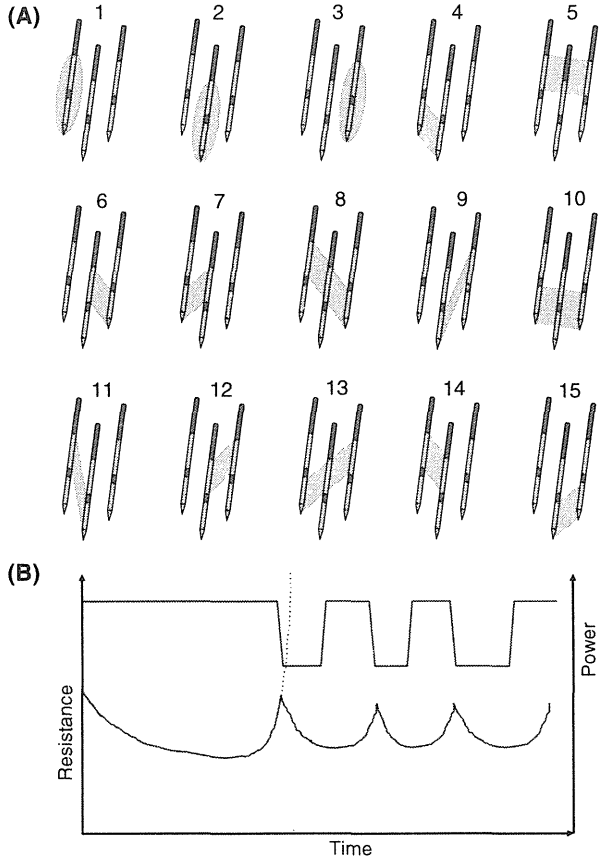

Fig. 3 When 3 applicators are employed, the high-frequency electrical current flows sequentially between 15 combinations of electrode pairs (**a**), and an image is generated of the automated control of the output by the resistance controlled automatic power (RCAP) function (**b**). RCAP is a function by which the degree of change in the electrical resistance among the electrodes (increase/decrease in slope) is monitored, and the high-frequency power output is automatically controlled

controls the high-frequency power (Fig. 3b). This function makes it possible to prevent unexpected rapid increases in electrical resistance resulting from tissue necrotization.

Patients

This clinical study was carried out based on the HCC treatment algorithm in the Scientific Data-based Clinical Practice Guidelines for Liver Cancer-2005 Version [18]. We enrolled adult male and female patients aged 20 years or older with primary or metastatic small liver cancers who had provided written informed consent. Target tumors were defined as nodular, numbering up to 3 lesions, each of which was 3 cm or less in diameter, or solitary lesions up to 4 cm in diameter. Exclusion criteria included a Child-Pugh grade of C, or platelet count below 50000/μl. Informed consent was obtained from 104 patients, of whom 96 were initially enrolled, but 5 withdrew consent before the trial started. The trial was therefore carried out in a total of 91 patients (112 treated lesions) with intention-to-treat (ITT) analysis, and 90 patients were eligible for the analysis of efficacy.

Patient details

Table 1 summarizes the data on the background characteristics of the 91 patients and 112 treated lesions treated in the study (73 patients had 1 lesion, 15 had 2, and 3 patients had 3 lesions; Table 1). The cohort consisted of 61 men and 30 women, and the mean age (±SD) was 69 ± 10 years; 84 patients had primary liver cancer, while 7 had metastatic liver cancer.

Study design

This prospective multicenter, collaborative, single-group, open-label study was conducted at 5 institutions between December 2008 and December 2009. The study protocol was approved by each center's institutional review board. The trial treatment period lasted from the acquisition of written informed consent through completion of the final treatment (maximum 3 treatments), in addition to a follow-up period from the day after the final examinations of the treatment period until the completion of examinations performed 24 weeks later. The non-inferiority of the CelonPOWER System was evaluated relative to the results obtained with a Cool-tip RF System in 2002–2003 [10].

Study methodology

Figure 4 shows the study procedures. During the treatment period, the following procedures were performed, in the order listed: registration of eligible patients, RFA treatment and examinations including computed tomography (CT) imaging, laboratory tests, and blood pressure measurement. The efficacy was evaluated from the extent of the necrotic area (tumor necrosis; TN) induced by ablation as measured on conventional and dynamic CT imaging. Additional ablation, up to a maximum of 3 sessions, was performed as necessary. The laboratory tests consisted of RBC count, WBC count, hemoglobin level, hematocrit, platelet count, prothrombin time (PT) activity, total bilirubin, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), and creatinine.

In the follow-up phase, at 10 ± 2 weeks (70 ± 14 days) and 24 ± 2 weeks (168 ± 14 days) following the day of the final RFA session, we performed CT imaging, laboratory tests, blood pressure measurement, measurement of alpha-fetoprotein (AFP), and measurement of protein induced by vitamin K absence or antagonist II (PIVKA-II). The CT images and tumor marker data were employed to assess the continuity of the therapeutic effect (TE) of the RFA treatment.

RFA procedure

The procedure with the CelonPOWER System device was similar to the procedure with the existing monopolar RFA devices. In all cases, the procedure was performed percutaneously under ultrasound guidance and local anesthesia.

Assessment of efficacy

TN was assessed using 5 grades, in accordance with the Criteria for Direct Effects of Liver Cancer Treatment (1994) [19]. Class V tumor necrosis (100 % TN) of liver cancer following the final RFA session was defined as "complete necrosis," and the percentage of patients achieving Class V TN was defined as the "complete necrosis rate" (CNR), the primary endpoint. The TN classification was used for short-term (during treatment) evaluation, and this was the only evaluation reported for the Cool-tip RF System in the marketing authorization holder's application for Japanese government approval. However, now the government demands not only short-term evaluation, but also long-term evaluation, for which such parameters as TE, overall response, and complete response (CR) are used.

The secondary endpoints of our study were the number of RFA sessions, the TE, and the overall assessment of the TE. The assessment of the immediate TE and the overall assessment of TE were performed in accordance with the General Rules for the Clinical and Pathological Study of Primary Liver Cancer (2008) [20]. The TE was classified as either CR (total necrosis and normalization of all tumor

Table 1 Patient background factors and lesion characteristics

Patients (n = 91)		Lesions (n = 112)	
Background factors	N (%)	Characteristics	N
Sex		Maximum dimension (cm)	
M	61 (67.0)	<1.0	22
F	30 (33.0)	1.1–2.0	69
Age (years)		2.1–3.0	17
31–40	1 (1.1)	3.1–4.0	4
41–50	4 (4.4)	Mean ± SD	
51–60	9 (9.9)	1.6 ± 0.7	
61–70	32 (35.2)	Subsegment	
71–80	34 (37.4)	S1	0
81–90	11 (12.1)	S2	6
Cancer		S3	9
Primary	84 (92.3)	S4	8
Metastatic	7 (7.7)	S5	18
Underlying disease		S6	20
Cirrhosis	63 (69.2)	S7	18
Chronic hepatitis	22 (24.2)	S8	33
None	6 (6.6)		
Child-Pugh classification			
Grade A	83 (91.2)		
Grade B	8 (8.8)		
Number of treated lesions			
1	73 (80.2)		
2	15 (16.5)		
3	3 (3.3)		
Previous treatment of primary disease			
Yes	40 (44.0)		
No	51 (56.0)		

markers), or others. In addition, ITT analysis was performed in regard to the cumulative local recurrence rate and the overall assessment of the TE.

Assessment of safety

The following safety endpoints were assessed in all 91 patients in whom the study was conducted: overall safety assessment, adverse events, device-related adverse events, device failure, laboratory test values, and blood pressure.

Statistical analysis

Statistical analysis was performed using a one-sided significance level of 2.5 % for the primary endpoint. In principle, a two-sided significance level of 5 % was used for the other endpoints to avoid data dispersion. The CNR (the primary endpoint) was calculated as the percentage of the total number of patients who achieved Class V TN, and its exact one-sided 97.5 % confidence interval was calculated. For the secondary endpoints, the variables and their ratios were compiled, and the basic statistics for the mean and standard deviation were calculated.

Results

Patients

Written informed consent was obtained from 104 patients, including the 96 patients in the study. The study was conducted in 91 of these patients, and treatment was completed in 90 patients. Eighty-eight of the 90 patients (excluding 2 TN4 patients) were followed up. Five patients discontinued the study during the follow-up period, leaving 83 patients who completed the follow-up period. Three patients were excluded because of unacceptable enrollment dates, so the final number of patients eligible for the efficacy analysis was 80.