been yet determined. Thus, we conducted this research with a large scale of samples.

Patients and methods

During 6 years, from January 2000 to December 2005, a total of 60,773 patients with primary liver cancer were prospectively registered bi-annually by the Liver Cancer Study Group of Japan (LCSGJ) throughout 800 medical institutions using a registration/questionnaire sheet with more than 180 questions. Among them, 53,008 patients were clinically diagnosed with HCC with multiple imaging modalities, tumor markers, and/or needle biopsy. Four thousand nine hundred sixty-six patients were selected in the current cohort study. Inclusion criteria were the following: TACE was performed in naïve patients as an initial treatment and any other therapy such as resection and local ablation was not performed during the first investigation period within at least 2 years. Exclusion criteria were: vascular invasion of the portal and hepatic veins, invasion of the biliary duct, extrahepatic spread and history of previous treatment for HCC.

HCC was diagnosed using ultrasonography (US), dynamic computed tomography (CT), magnetic resonance imaging (MRI), and/or pathologically by biopsy specimens (3.2%). Abnormal elevation of tumor markers was also referred: alpha-fetoprotein (AFP) >400 ng/ml (normal, <20) and des-gamma carboxyl prothrombin (DCP) >100 mAU/ml (normal, <40). Typical HCC was depicted as hyper-attenuation in arterial phase and hypo-attenuation or wash-out in delayed phase (around 3 min after the beginning of contrast injection) of dynamic CT and on dynamic MRI. If the tumor showed an atypical profile and was larger than 2 cm in diameter, further examination was recommended as follows: angiography, combination of CT and angiography, MRI with super-paramagnetic iron oxide, CE-US with micro-bubble (Levovist, Bayer Schering Pharma, Germany), and/or needle biopsy. If the tumor was less than 2 cm, a follow up study with US was recommended [6]. The extrahepatic metastases were routinely examined by CT, US, and chest X-ray.

The distribution of background factors of patients with TACE is shown in Table 1. The study population predominantly consisted of patients older than 60 years [n = 4205 (85%)] and among them 3369 were male patients (68%). The proportion of Child–Pugh A/B/C was 69% (n = 3229 patients), 28% (n = 1296), and 4% (n = 167), respectively. 3479 patients (73%) were positive for hepatitis C virus antibody and 449 were positive for hepatitis B virus surface antigen. The maximum tumor size was ≤2 cm in diameter for 32% and ≤3 cm for 56% of tumors. The mean diameter was 3.8 ± 3.5 (standard deviation, SD) cm. The tumor number was one in 2252 patients (46%), two in 1003, three in 565, and more than four in 1092. 1868 patients (40%) had a normal AFP value and 900 had more than 401 ng/ml. 2128 patients (52%) had a DCP value ≤100 mAU/ml.

According to the TNM stage revised by the LCSGJ in 2000 [7], 836 patients were in stage I, 2070 (43%) in stage II, and 1887 in stage III. The embolization area was less than one segment in 1589 patients (33%), equal to or more than one segment to less than one lobe in 2134 (44%), and the whole liver in only 247 patients (5%). Hypervascular HCC accounted for 98% (n = 4787 patients) and non-hypervascular HCC for 2% (n = 100). Mean bilirubin value was 1.1 ± 0.9 mg/dl (SD). Performance status (PS) according to Eastern Cooperative Oncology Group scale was PS0 in 1485 (80%) patients, PS1 in 298, PS2 in 48, PS3 in 23, and PS4 in 2 out of 1856 patients, namely 99% of patients, which were available during the last two years (January, 2004 to December, 2005) of the present study, were in PS0–2.

In most patients, the catheter tip was advanced at the nearest site of the feeding artery as possible. The emulsion of the anticancer agent and lipiodol followed by gelatin sponge particles was injected under X-ray monitoring. The dose of emulsion and particles of embolic materials was determined mainly based on the tumor size and extension. The anticancer agent used was epirubicin hydrochloride in 1490 patients (74%), doxorubicin hydrochloride in 191 patients, mitomycin C in 190 patients, and cisplatin and zinostatin stimalamer (SMANCS) in 72 patients each, for a total of 2015 patients with a mean dose of lipiodol of 4.8 ± 3.0 ml (SD), which data were available during the last two years (January, 2004 to December, 2005). The patients underwent dynamic CT or MRI with AFP and DCP measurement every three to four months, and repeated TACE was determined when local recurrence, intrahepatic metastases and/or de novo HCC was found.

To analyze the survival rate, all patients in Child–Pugh A or B were divided in four groups depending on tumor number (single, two, three, and more than four lesions). Each group was subsequently subdivided in four subgroups based on tumor size; ≤2, 2.1 to 3.0, 3.1 to 5.0, and ≥5.1 cm in diameter. Patients in Child–Pugh C were excluded from this analysis due to their small number (n = 167). The survival rate was calculated from the date of TACE to December 31, 2005. Patient's death was the endpoint irrespective of the cause of death. The mean follow up period was 1.6 ± 1.3 years (SD). TACE-related death was designated as death within 30 days after the initial TACE.

Treatment algorithm proposed by Japanese guidelines

The treatment algorithm proposed by Japanese guidelines [6] has six treatments determined by three factors: degree of liver damage [7], number of tumors, and tumor diameter (Fig. 1). For patients with liver damage A or B, four treatments are recommended: resection for single tumor or local ablation for single tumor ≤2 cm and liver damage B; resection or ablation for 2 or 3 tumors ≤3 cm; resection or TACE for 2 or 3 tumors >3 cm; TACE or hepatic arterial infusion chemotherapy for more than 4 tumors. For patients with liver damage C, liver transplantation for 1 to 3 tumors ≤3 cm or single tumor ≤5 cm as indicated by the Milan criteria [8], and palliative care for ≥4 tumors are recommended. In the present study, Child–Pugh class was adopted instead of degree of liver damage because the former is widely used to evaluate liver function, especially for candidates to TACE.

The executing rate of TACE was calculated with the following formula: number of patients stratified to TACE in treatment algorithm divided by a total number of patients who actually received TACE × 100 (%). The adequacy of treatment algorithm for TACE was validated when the survivals of patients stratified to TACE group (for 2 or 3 lesions >3 cm or more than 4 lesions) and those of patients stratified to non-TACE group (such as resection and ablation for single lesion or 2 or 3 lesions ≤3 cm) could be discriminated.

Statistical analysis

The survival rate was obtained by the Kaplan–Meier method and compared by the log-rank test in Tables 1, 2A and B, and 3. The multivariate analysis was performed with the Cox's proportional hazard model. All variables, except for one of the embolization area of the liver due to the factor obtained following TACE therapy, with p value less than 0.05 on univariate analysis, were subjected to multivariate analysis. All significance tests were two-tailed, and p value less than 0.05 was considered statistically significant. All statistical analyses were carried out with the Statistical Analysis System (SAS) version 8.02 (SAS Inc., Cary, NC).

Results

Survival rates

For overall survival of the 4966 patients who underwent TACE, the median, and 1-, 2-, 3-, 4- and 5-year survival rates were 3.3 years (40 months) and 87%, 70%, 55%, 42%, and 34%, respectively (Fig. 2). The 3- and 5-year survival in Child–Pugh A, B, and C was 61% and 40%; 43% and 22%; 23% and 0%, respectively (Table 1).

Patient characteristics analyzed by univariate and multivariate analyses

The univariate analysis revealed that there was a significant difference between the following seven variables ($p = 0.0001$); Child–Pugh class, maximum tumor size, number of lesions, AFP, DCP, TNM stage, and extent of embolization area (Table 1).

The multivariate analysis showed that the following five variables were independent predictors in trial 1; Child–Pugh class, tumor size, number of lesions, AFP, and DCP (Supplementary Table 1). In trial 2, where tumor size and number of lesions in trial 1 were replaced by TNM stage, four variables were independent predictors: Child–Pugh class, TNM stage, AFP, and DCP.

Survival rates of patients stratified to four groups divided by lesion number and to four subgroups subdivided by lesion size

In Child–Pugh A patients (n = 3194), the overall median and 3-year survival rate in four groups divided by tumor number: single, two, three, and more than 4 lesions, were 5.4 years and 73%, 3.8 years and 59%, 3.1 years and 52%, and 2.8 years and

Table 1. Distribution of background factors and results of the univariate analysis in 4966 patients with hepatocellular carcinoma who underwent transcatheter arterial chemoembolization with lipiodol.

Background factors	No. of patients	Proportion (%)	Survivals (%)				*p*	Hazard ratio (95% CI)
			1-yr	3-yr	4-yr	5-yr		
Age, yr							0.88	
<60	756	15	85	56	43	39		Ref.
≥60	4205	85	87	55	42	33		1.01 (0.88, 1.16)
Gender							0.40	
M	3369	68	86	54	42	35		1.05 (0.93, 1.18)
F	1597	32	89	56	42	33		Ref.
Child-Pugh classification							0.0001	
A	3229	69	90	61	49	40		Ref.
B	1296	28	82	43	27	22		1.81 (1.62, 2.04)
C	167	4	69	23	12	-		3.05 (2.44, 3.81)
HBV and HCV							0.50	
HCV Ab positive	3479	73	87	55	41	34		1.01 (0.87, 1.18)
HBs Ag positive	449	9	84	53	37	35		1.11 (0.90, 1.38)
Both positive	89	2	89	58	54	43		0.83 (0.56, 1.25)
Both negative	768	16	86	56	44	32		Ref.
Maximum tumor size (cm)							0.0001	
≤2	1549	32	93	65	50	42		Ref.
2.1-3	1178	24	89	53	42	35		1.38 (1.19, 1.60)
3.1-5	1291	27	85	52	37	29		1.62 (1.41, 1.87)
≥5.1	811	17	77	44	34	23		2.19 (1.88, 2.56)
No. of lesions							0.0001	
1	2252	46	91	66	53	45		Ref.
2	1003	20	88	55	42	34		1.35 (1.17, 1.56)
3	565	12	86	45	27	20		1.77 (1.50, 2.08)
≥4	1092	22	79	39	30	20		2.18 (1.91, 2.48)
Alpha-fetoprotein (ng/ml)							0.0001	
≤20	1868	40	92	64	50	44		Ref.
21-200	1613	34	89	55	40	30		1.38 (1.21, 1.57)
201-400	311	7	82	45	33	29		1.73 (1.40, 2.14)
401-1000	309	7	81	43	32	21		2.07 (1.68, 2.55)
≥1001	591	13	72	38	32	23		2.49 (2.13, 2.92)
Des-gamma carboxy-prothrombin (mAU/ml)							0.0001	
≤100	2128	52	92	65	52	40		Ref.
101-299	599	15	88	52	41	32		1.50 (1.26, 1.78)
300-499	245	6	84	49	27	24		1.93 (1.54, 2.42)
500-999	294	7	82	50	33	20		1.89 (1.51, 2.36)
≥1000	794	20	76	38	26	18		2.52 (2.18, 2.91)
TNM stage							0.0001	
I (T1N0M0)	836	17	93	72	59	51		Ref.
II (T2N0M0)	2070	43	90	60	46	37		1.51 (1.27, 1.80)
III (T3N0M0)	1887	39	81	42	30	22		2.60 (2.19, 3.09)
Extent of embolization							0.0001	
<one segment	1589	33	90	64	51	44		Ref.
1 seg. ≤ to <1 lobe	2134	44	87	55	41	32		1.33 (1.17, 1.51)
1 lobe ≤ to <whole liver	873	18	85	47	32	23		1.67 (1.43, 1.94)
Whole liver	247	5	74	37	29	-		2.27 (1.85, 2.80)

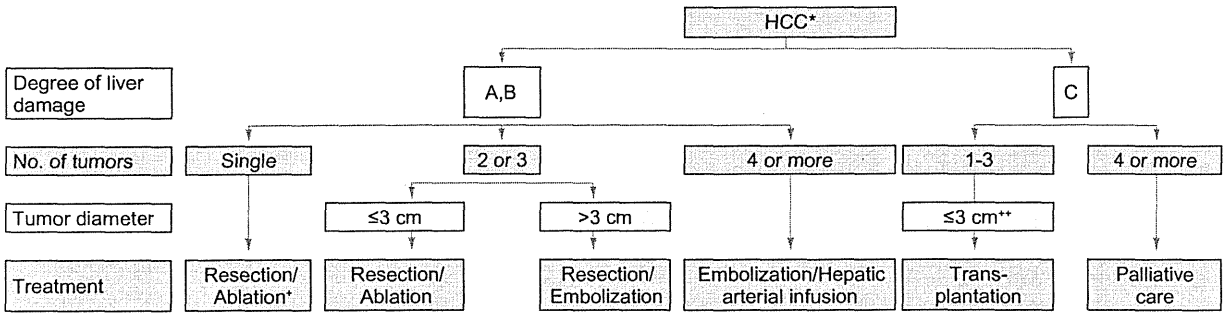

Fig. 1. Treatment algorithm for HCC proposed by Japanese guidelines. (+) shows local ablation for single lesion ≤2 cm in patients with liver damage B. (++) means liver transplantation for no more than 3 lesions ≤3 cm or single lesion ≤5 cm. The asterisk shows that for patients with vascular invasion and liver damage A, hepatectomy, TACE or hepatic arterial infusion chemotherapy may be recommended, while chemotherapy is an option for patients with extrahepatic metastasis.

46%, respectively ($p = 0.0001$, Table 2A). The survival rate of four subgroups subdivided by tumor size from ≤2 cm to ≥5.1 cm decreased as the lesion size increased in all ($p = 0.04$ to $p = 0.0001$) but one group with 3 lesions ($p = 0.07$). The highest 3-year survival was 80% in patients with single lesion ≤2 cm, and the lowest 3-year survival was 30% in patients with more than 4 lesions ≥5.1 cm.

In Child–Pugh B (n = 1284), the overall median and 3-year survival rate of four groups were 3.1 years and 53%, 2.8 years and 49%, 2.0 years and 24%, and 1.9 years and 22%, respectively ($p = 0.0001$, Table 2B). The survival rate of four subgroups divided by tumor size in each group decreased as the lesion size increased in all ($p = 0.01$ to $p = 0.0004$) but one group with single lesion ($p = 0.49$). The highest 3-year survival was 65%, found in patients with 2 lesions ≤2 cm, and the lowest was 0% in patients with three lesions ≥5.1 cm.

Validation of the treatment algorithm proposed by the Japanese guidelines

Of 3168 patients with TACE in Child–Pugh A, 1475 were stratified to resection or ablation therapy for single lesion in the treatment algorithm (Fig. 1), 506 to resection or ablation for 2 or 3 lesions ≤3 cm, 463 to resection or TACE for 2 or 3 lesions >3 cm, and 724 to TACE or hepatic arterial infusion chemotherapy for ≥4 lesions (Table 3). The median and 3-year survival rates of the corresponding four treatments were 5.4 years and 73%, 3.5 years and 59%, 3.4 years and 55%, and 2.8 years and 46%, respectively, with a significant difference ($p = 0.0001$). The comparisons of the survival curves between two treatment groups showed a significant difference in all ($p = 0.013$ to $p = 0.0001$) but one comparison between treatments for 2 or 3 lesions ≤3 cm and for 2 or 3 lesions >3 cm ($p = 0.06$) (Fig. 3). Namely, survival discrimination was feasible between one of two TACE treatments for >4 lesions and non-TACE therapies such as resection or ablation.

Similarly, 1274 patients with Child–Pugh B were stratified to four treatment categories (Table 3). The median and 3-year survivals of these treatments from single to ≥4 lesions were 3.1 years and 53%, 2.8 years and 49%, 1.7 years and 30%, and 1.9 years and 22%, respectively, with a significant difference ($p = 0.0001$). The comparisons of survival curves between two treatment groups showed a significant difference in all ($p = 0.0001$) but two comparisons; single lesion *vs.* 2 or 3 lesions ≤3 cm ($p = 0.79$) and 2 or 3 lesions >3 cm *vs.* ≥4 lesions ($p = 0.84$) (Fig. 4). Namely, survival discrimination was feasible between two TACE therapy groups, i.e., 2 or 3 lesions >3 cm and ≥4 lesions and two non-TACE groups.

The executing rate of TACE was 37% in both Child–Pugh A (1187/3168 patients) and B (467/1274).

TACE-related mortality rate

After the initial TACE, treatment-related death occurred in 19 (0.38%) out of 4966 patients. The breakdown of the cause of death was cancer in 5 patients (26%), hepatic failure in 3 (16%), rupture of esophago-gastric varices in one patient, intra-peritoneal rupture of HCC in another patient, and other causes in 9 patients. Ten patients were in Child–Pugh A, 8 were in class B and one was in class C.

Discussion

The present study demonstrates that the overall median and 3-, and 5-year survival rates of TACE were 3.3 years (40 months), 55%, and 34%, respectively, and were better than those previously reported by the LCSGJ (34 months, 47%, and 26% [9]), mainly due to exclusion criteria of vascular invasion in the current study. The multivariate analysis revealed that five variables were independent predictors in trial 1: Child–Pugh class, tumor size, tumor number, AFP, and DCP; and four variables in trial 2, where tumor size and tumor number were replaced by TNM stage. These results are similar to those of a previous study [9] other than Child–Pugh class instead of degree of liver damage and DCP value were newly adopted.

There was an inverse correlation between tumor number and overall survival of patients with TACE therapy ($p = 0.0001$) in both Child–Pugh A and B (Tables 2A and B) as well as between tumor diameter and survival in all but one group, each in Child–Pugh A and B. Namely, the fewer the tumor number and the smaller the tumor size, the better the survival rates. The best 3-year survival (80%) was found in patients with a single HCC ≤2 cm in Child–Pugh A, and the worst 3-year survival (0%) in patients with three lesions ≥5.1 cm in class B. However, in clinical practice, the best survivor with TACE is not recommended to TACE but to resection or local ablation due to relatively higher 3-year survival rates, 90% and 85%, respectively [3]. The current study has revealed a wide range of survival rates for patients with

Table 2. The overall survivals of four groups divided by tumor number and survivals of four subgroups divided by tumor size in patients who underwent TACE. (A) Child-Pugh A (n = 3194 patients). (B) Child-Pugh B (n = 1284).

A

Group/ subgroup	No. of patients	Survival (%) 1-yr	3-yr	4-yr	5-yr	Median (yr)	p	Hazard ratio (95% CI)
Single lesion								
Overall*	1475	93	73	62	52	5.4		
≤2 cm	546	97	80	73	65	-	0.0001	Ref.
2.1-3.0	353	92	71	56	36	4.5		1.88 (1.36, 2.62)
3.1-5.0	328	92	66	53	46	4.6		1.97 (1.42, 2.75)
≥5.1	219	86	66	48	-	4.2		2.38 (1.64, 3.46)
Two lesions								
Overall*	634	91	59	47	40	3.8		
≤2 cm	178	97	64	49	42	3.9	0.04	Ref.
2.1-3.0	144	91	50	39	-	2.8		1.57 (1.01, 2.44)
3.1-5.0	190	90	66	47	39	4.0		1.23 (0.80, 1.90)
≥5.1	104	84	53	45	38	4.1		1.94 (1.19, 3.15)
Three lesions								
Overall*	361	90	52	33	24	3.1		
≤2 cm	102	92	65	30	-	3.6	0.07	Ref.
2.1-3.0	82	95	51	32	-	3.1		1.16 (0.68, 1.99)
3.1-5.0	111	94	48	38	-	3.0		1.30 (0.80, 2.10)
≥5.1	58	73	35	-	-	2.2		2.02 (1.19, 3.45)
More than 4 lesions								
Overall*	724	82	46	37	25	2.8		
≤2 cm	168	92	59	54	44	4.4	0.0001	Ref.
2.1-3.0	137	83	54	51	32	4.0		1.47 (0.97, 2.25)
3.1-5.0	207	82	43	25	16	2.5		2.00 (1.38, 2.90)
≥5.1	190	74	30	18	-	1.7		2.89 (2.01, 4.17)

B

Group/ subgroup	No. of patients	Survival (%) 1-yr	3-yr	4-yr	5-yr	Median (yr)	p	Hazard ratio (95% CI)
Single lesion								
Overall*	568	87	53	34	30	3.1		
≤2 cm	213	89	56	33	24	3.3	0.49	Ref.
2.1-3.0	169	88	50	44	39	2.9		1.00 (0.69, 1.44)
3.1-5.0	132	87	54	20	-	3.1		1.24 (0.83, 1.86)
≥5.1	47	78	49	-	-	2.5		1.40 (0.80, 2.47)
Two lesions								
Overall*	276	83	49	34	-	2.8		
≤2 cm	86	93	65	48	-	4.0	0.0004	Ref.
2.1-3.0	70	93	43	21	-	2.3		1.86 (1.04, 3.34)
3.1-5.0	82	69	41	27	-	2.1		2.49 (1.47, 4.21)
≥5.1	31	58	-	-	-	1.5		3.66 (1.83, 7.32)
Three lesions								
Overall*	150	77	24	14	-	2.0		
≤2 cm	40	89	28	19	-	2.0	0.005	Ref.
2.1-3.0	43	76	30	-	-	2.3		1.09 (0.50, 2.35)
3.1-5.0	41	73	34	17	-	1.8		1.31 (0.65, 2.64)
≥5.1	23	62	-	-	-	1.4		3.16 (1.49, 6.72)
More than 4 lesions								
Overall*	290	72	22	10	-	1.9		
≤2 cm	57	90	32	24	-	2.0	0.01	Ref.
2.1-3.0	68	75	17	8	-	2.1		1.53 (0.85, 2.76)
3.1-5.0	89	73	25	-	-	2.0		1.54 (0.88, 2.69)
≥5.1	65	54	-	-	-	1.2		2.55 (1.43, 4.56)

*A significant difference was demonstrated in overall survival among four groups ($p = 0.0001$).

TACE, mainly because of the heterogeneity of the population, therefore it would be helpful for candidates to determine chemoembolization as tailor-made treatment of choice; this is particularly suitable for patients averse to curative therapy and with severely associated diseases, or elderly patients.

The executing rate of patients who actually had undergone TACE and were stratified to TACE in the treatment algorithm was 37% in both Child-Pugh A and B. Namely, the remaining 63% of patients satisfied the criteria of resection or local ablation (non-TACE therapy), which could suggest the possible increase of survival in these patients, if they underwent resection or local ablation. The reason for the lower executing rate might be the less publicity in which the guideline was published one year after the completion of this 6-year study.

The discrimination of patients' survival was feasible in this treatment algorithm between TACE and non-TACE therapies in Child-Pugh B and in part in class A. Further studies are needed to validate the suitability of these guidelines using patients who underwent resection or local ablation and are stratified to four treatments like in the TACE study.

To our knowledge, the present study is the first report to clarify the median and 3- and 4-year survivals of patients treated by TACE and stratified in the four treatments recommended by the Japanese guidelines in Child-Pugh A and B, separately. Interestingly, Llovet *et al.* [10] stated that chemoembolization improved median survival up to 19–20 months in intermediate stage of BCLC classification, which is similar to our results; 1.7 years (20 months) in patients with TACE for 2 or 3 lesions >3 cm and

Table 3. Survival rates of patients treated with TACE stratified to four treatment categories recommended by Japanese guidelines in Child–Pugh A and B.

Criteria of treatment	No. of patients	Survival (%)				Median	*p*	Hazard ratio (95% CI)
		1-yr	3-yr	4-yr	5-yr	(yr)		
Child-Pugh A	3168							
Single lesion	1475	93	73	62	52	5.4	0.0001	Ref.
2-3 lesions, ≤3 cm	506	94	59	40	36	3.5		1.45 (1.18, 1.78)
2-3 lesions, >3 cm	463	87	55	44	31	3.4		1.82 (1.48, 2.25)
≥4 lesions	724	82	46	37	25	2.8		2.39 (2.01, 2.84)
Child-Pugh B	1274							
Single lesion	568	87	53	34	30	3.1	0.0001	Ref.
2-3 lesions, ≤3 cm	239	90	49	33	-	2.8		1.04 (0.79, 1.36)
2-3 lesions, >3 cm	177	68	30	19	-	1.7		2.11 (1.62, 2.75)
≥4 lesions	290	72	22	10	-	1.9		2.17 (1.72, 2.74)

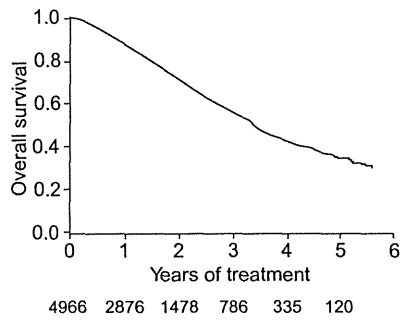

Fig. 2. Overall survival rate of 4966 HCC patients who underwent TACE.

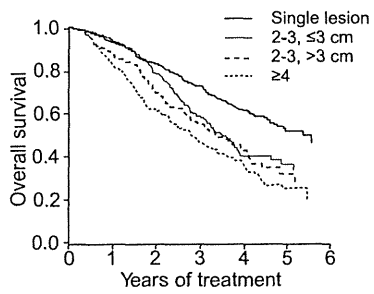

Fig. 3. The survival curves of 3168 patients in Child–Pugh A stratified to four treatment groups according to Japanese guidelines. Overall, there was a significant difference ($p = 0.0001$). There was also a significant difference between two treatment groups except for one; 2 or 3 lesions ≤3 cm *vs.* 2 or 3 lesions >3 cm ($p = 0.06$).

1.9 years (23 months) in those for ≥4 lesions in Child–Pugh B (Table 3). If the criteria for TACE are similar in the intermediate stage of the BCLC staging system [5] and in the treatment algorithm of the Japanese guidelines: equal to or more than 4 lesions and/or 2 or 3 lesions >3 cm [11], the current data will be useful to compare the survival outcomes of TACE in the East and West. The survival rates of our study will be also used as reference data

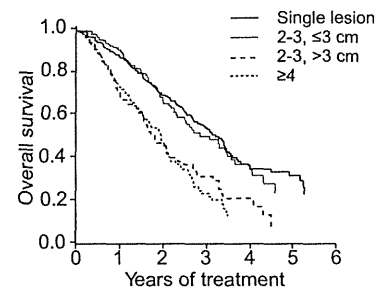

Fig. 4. The survival curves of 1274 patients in Child–Pugh B stratified to four treatment groups. Overall, a significant difference was seen ($p = 0.0001$). A significant difference was observed between two groups except for two; single lesion *vs.* 2 or 3 lesions ≤3 cm, and 2 or 3 lesions >3 cm *vs.* ≥4 lesions.

when clinical trials of TACE with or without anti-angiogenic drugs are newly designed [12,13].

The treatment-related mortality rate was 0.38%, which was slightly improved compared to that of our previous study of 0.5% [9], and much better than that of 2.4% reported by a systematic review [14]. The improvement is mainly attributable to the exclusion criteria of vascular or biliary duct invasion and the decreased proportion of Child–Pugh C patients, from 10% [9] to 4%.

As a limitation of this study, the session numbers of TACE per patient and dosage of anticancer agent used at initial TACE were not available due to lack of inclusion in the questionnaire sheet. Our patients received different TACE protocols for anticancer agent. Given that the large majority of patients were treated with epirubicin or doxorubicin, it could be worth limiting the analysis to these patient cohorts.

In conclusion, the overall median and 3- and 5-year survival of TACE were 3.3 years, 55% and 34% in 4966 HCC patients without vascular invasion and extrahepatic spread. The tumor number, size, liver function, AFP, and DCP were independent predictors. These results will be helpful for physicians to select chemoembolization as optimal therapy for their patients, especially when curative treatment is contraindicated due to severely associated disease and/or aging. The treatment algorithm of the Japanese guidelines might be appropriate to discriminate patient survival

with non-TACE from TACE therapy in Child–Pugh B and in part in A. The survival rates of patients stratified to TACE in these guidelines will be useful for comparing the outcome of TACE in the East and West, and for designing new clinical trials for TACE with and without a novel molecular targeted agent as reference data.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2011.10.021.

References

[1] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
[2] Bruix J, Llovet JM. Prognostic prediction and treatment strategy in hepatocellular carcinoma. Hepatology 2002;35:519–524.
[3] Ikai I, Kudo M, Arii S, Omata M, Kojiro M, Sakamoto M, et al. Report of the 18th follow up survey of primary liver cancer in Japan. Hepatol Res 2010;40:1043–1059.
[4] Matsui O, Kadoya M, Yoshikawa J, Gabata T, Arai K, Demachi H, et al. Small hepatocellular carcinoma: treatment with subsegmental transcatheter arterial embolization. Radiology 1993;188:79–83.
[5] Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001;35:421–430.
[6] Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, et al. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res 2008;38:37–51.
[7] The Liver Cancer Study Group of Japan. The general rules for the clinical and pathological study of primary liver cancer. 2nd English ed. Tokyo, Japan: Kanehara & Co., Ltd.; 2003.
[8] Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med 1996;334:693–699.
[9] Takayasu K, Arii S, Ikai I, Omata M, Okita K, Ichida T, et al. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. Gastroenterology 2006;131:461–469.
[10] Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008;100:698–711.
[11] Takayama T. Hepatocellular carcinoma. In: Clavien PA, editor. Malignant liver tumors: current and emerging therapies. London: Wiley-Blackwell; 2010. p. 317–323.
[12] Sergio A, Cristofori C, Cardin R, Pivetta G, Ragazzi R, Baldan A, et al. Transcatheter arterial chemoembolization (TACE) in hepatocellular carcinoma (HCC): the role of angiogenesis and invasiveness. Am J Gastroenterol 2008;103:914–921.
[13] Lencioni R, Zou J, Leberre M, Meinhardt G, Voliotis D, Bruix J, et al. Sorafenib (SOR) or placebo (PL) in combination with transarterial chemoembolization (TACE) for intermediate-stage hepatocellular carcinoma (SPACE). ASCO Meeting Abstracts 2010;28:TPS178.
[14] Marelli L, Stigliano R, Triantos C, Senzolo M, Cholongitas E, Davies N, et al. Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. Cardiovasc Intervent Radiol 2007;30:6–25.

Tolerability of adjuvant chemotherapy with S-1 after curative resection in patients with stage II/III gastric cancer

HIRONORI TSUJIMOTO, HIROYUKI HORIGUCHI, SHUICHI HIRAKI, YOSHIHISA YAGUCHI, RISA TAKAHATA, ISAO KUMANO, KAZUMICHI YOSHIDA, YUSUKE MATSUMOTO, SATOSHI ONO, JUNJI YAMAMOTO and KAZUO HASE

Department of Surgery, National Defense Medical College, Tokorozawa 359-8513, Japan

Received April 24, 2012; Accepted August 13, 2012

DOI: 10.3892/ol.2012.882

Abstract. The results of the Japan Clinical Oncology Group trial demonstrated that adjuvant chemotherapy with S-1 for stage II/III gastric cancer is effective and suggested that this therapy should be adopted as the standard treatment following curative D2 gastric dissection. We reviewed treatment outcomes in 58 consecutive patients who received adjuvant therapy with S-1 for stage II/III gastric cancer following curative D2 dissection; the standard dosage used was determined on the basis of the patient body surface area. Twenty-four patients (41.3%) discontinued treatment before 12 months. Patients who completed 12 months of adjuvant therapy with S-1 were younger and more frequently treated by senior doctors (>15 years of experience) than those who did not. However, no differences existed in pathological features and surgical procedures between groups. Overall survival and relapse-free survival were better in patients who completed 12 months of adjuvant therapy with S-1. Fatigue and nausea were associated with discontinuation of S-1 treatment. In conclusion, immediately after surgery, fatigue and gastrointestinal symptoms of ≤ grade 2 may have a major impact on treatment compliance. Prior to the commencement of S-1 administration, both patients and doctors should be made completely aware of the toxicity, compliance and efficacy issues associated with this adjuvant therapy.

Introduction

S-1 is an oral anticancer preparation composed of a mixture of tegafur [FT, a prodrug of 5-fluorouracil (5-FU)], 5-chloro-2,4-dihydroxypyridine (CDHP, a biochemical modulator that inhibits 5-FU biodegradation) and potassium oxonate (Oxo, added to reduce the gastrointestinal toxicity of 5-FU) (1-3). In the two registration phase II studies in Japan, the rate of response to treatment with S-1 alone exceeded 40% in patients with advanced or recurrent gastric cancer (4,5). The Japan Clinical Oncology Group (JCOG) conducted a randomized prospective controlled study to evaluate the efficacy of single-agent S-1 as adjuvant therapy for patients with stage II/III (Japanese Classification of Gastric Carcinoma, JCGC) (6) gastric cancer following curative D2 dissection (7). When the final analysis was performed in September 2006, 3-year overall survival (OS) was 80.5% for S-1 treated patients and 70.1% for patients who underwent surgery alone. The hazard ratio for death in S-1 treated patients was 0.68 (P=0.0024). The results of this trial demonstrated that adjuvant chemotherapy with S-1 for stage II/III gastric cancer is effective and suggested that this therapy should be adopted as the standard treatment following curative D2 gastric dissection (8).

To investigate the tolerability of adjuvant chemotherapy with S-1 for stage II/III gastric cancer following curative D2 dissection, we reviewed treatment outcomes in patients receiving this adjuvant therapy.

Materials and methods

Patients. Between August 2007 and July 2010, 283 patients underwent gastrectomy for adenocarcinoma of the stomach with curative intent at the National Defense Medical College Hospital (Tokorozawa, Saitama, Japan). Of these, 64 patients (41-84 years old) had pathological stage II/III disease according to the JCGC (6). All patients were informed of the efficacy of the adjuvant chemotherapy trial of S-1 for gastric cancer (ACTS-GC) and provided their consent to the study (7).

Treatment regimen. S-1 was orally administered twice daily for 4 weeks, followed by a 2-week rest. This schedule was repeated every 6 weeks for 12 months until tumor recurrence, observation of unacceptable toxicity levels or refusal by the patient to undergo further treatment. Dosages were assigned according to the patient body surface area: <1.25 m^2, 80 mg/day; 1.25-1.5 m^2, 100 mg/day; and ≥1.5 m^2, 120 mg/day. Dosage modification and treatment interruption were performed according to the protocol in the registration trial (5,7). The dose or treatment schedule was modified at the physician's discretion according to the toxicity profiles. In

Correspondence to: Dr Hironori Tsujimoto, Department of Surgery, National Defense Medical College, 3-2 Namiki, Tokorozawa 359-8513, Japan
E-mail: tsujihi@ndmc.ac.jp

Key words: gastric cancer, adjuvant therapy, tolerability

Table I. Demographic and clinicopathological data.

Clinicopathological data	Total	Completed	Discontinued	P-value
Number of patients	58	34	24	
Age (years)	63.4±8.0	61.4±83.4	66.3±6.5	0.02
Gender				
Male	43 (74.1%)	24 (70.6%)	19 (79.2%)	0.42
Female	15 (25.9%)	10 (29.4%)	5 (20.8%)	
Histological classification				
Intestinal	23 (39.7%)	12 (35.3%)	11 (45.8%)	0.52
Diffuse	32 (55.2%)	19 (55.9%)	13 (54.2%)	
Adenosquamous	2 (3.4%)	2 (5.9%)	0 (0.0%)	
Tumor depth				
T2	24 (41.4%)	15 (44.1%)	9 (37.5%)	0.83
T3	32 (55.2%)	18 (52.9%)	14 (58.3%)	
T4	2 (3.4%)	1 (2.9%)	1 (4.2%)	
Lymph node metastasis				
N0	10 (17.2%)	6 (17.6%)	4 (16.7%)	0.85
N1	27 (46.6%)	15 (44.1%)	12 (50.0%)	
N2	21 (36.2%)	13 (38.2%)	8 (33.3%)	
Stage				
II	25 (43.1%)	15 (44.1%)	10 (41.7%)	0.18
IIIA	18 (31.0%)	8 (23.5%)	10 (41.7%)	
IIIB	15 (25.9%)	11 (32.4%)	4 (16.7%)	
Type of gastrectomy				
Total	30 (51.7%)	20 (58.8%)	10 (41.7%)	0.24
Distal	28 (48.3%)	14 (41.2%)	14 (58.3%)	
Reconstruction				
Billroth I	22 (37.9%)	11 (32.4%)	11 (45.8%)	0.48
Billroth II	3 (5.2%)	2 (5.9%)	1 (4.2%)	
Roux en Y	33 (56.9%)	21 (61.8%)	12 (50.0%)	
Cholecystectomy				
Yes	24 (41.4%)	14 (41.2%)	10 (41.7%)	0.95
No	34 (58.6%)	19 (55.9%)	14 (58.3%)	
Splenectomy				
Yes	20 (34.5%)	12 (35.3%)	8 (33.3%)	0.81
No	38 (65.5%)	22 (64.7%)	16 (66.7%)	
Doctor in charge				
Junior (≤15 yrs)	25 (43.1%)	11 (32.4%)	14 (58.3%)	0.04
Senior (>15 yrs)	33 (56.9%)	23 (67.6%)	10 (41.7%)	
Total amount of S-1 (mg)	16495.4±8851.9	23146.7±3335.6	7350.0±4954.9	<0.0001

principle, if patients had hematological toxic effects of grade 3 or 4 or non-hematological toxic effects of ≥ grade 2, their daily dose was reduced and/or their schedule was changed from a 4-week administration followed by a 2-week rest, to a 2-week administration followed by a 1-week rest.

Measures. If no gross residual disease was evident at the time of surgery and the resection margins were tumor-free on histological examination, surgery was considered curative. Pathological findings in gastric cancer patients were described on the basis of the JCGC (6). Adverse reactions were evaluated according to the common toxicity criteria of the National Cancer Institute, version 3.0 (http://ctep.cancer.gov).

OS was measured from the date of resection to the date of mortality from any cause. Relapse-free survival was measured from the date of resection to the date when relapse was evident by computed tomography, gastrointestinal endoscopic examination, abdominal ultrasonography, upper gastrointestinal series and/or positron emission tomography. Data for the patients who survived were censored in our survival analyses.

Table II. Adverse reactions to adjuvant therapy with S-1 among the 58 patients included in this study.

	No. of patients				Percentage (%)	
Adverse reaction	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1 or 2	Grade 3 or 4
Leukopenia	5	3	3	-	14.0	5.3
Anemia	26	3	-	-	50.9	0
Elevated t-bil level	4	2	-	-	10.5	0
Stomatitis	5	2	-	-	12.3	0
Anorexia	20	1	1	-	36.8	1.8
Nausea	6	2	-	-	14.0	0
Diarrhea	8	6	1	-	24.1	1.8
Skin lesions	8	1	-	-	15.5	0
Fatigue	8	3	-	-	19.3	0
Watering or dry eye	7	6	-	-	22.8	0

t-bil, total bilirubin.

The medication completion rate was measured from the date of treatment commencement to the date of treatment discontinuation. Data for patients in whom S-1 treatment was discontinued due to tumor recurrence or mortality were censored in this analysis. All patients were observed at our hospital or outpatient clinic at 2- to 4-week intervals up to 12 months after surgery, 3- to 4-month intervals during the 2 years of the study and every 6 or 12 months thereafter for 3 years.

Statistical analysis. Statistical calculations were performed using StatView version 5.0 (SAS Institute, Inc., Cary, NC, USA). Data are expressed as the means ± SEM. Statistical analyses were performed using the Mann-Whitney U test or Chi-square test with Fisher's exact test, as appropriate. Survival and medication completion rates were calculated using the Kaplan-Meier method and the significance of the difference was determined by a log-rank test. $P<0.05$ was considered to indicate a statistically significant result.

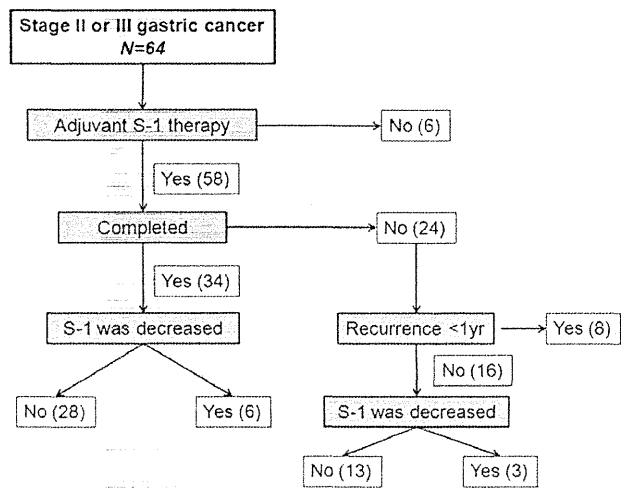

Figure 1. Flowchart of the treatment outcomes of adjuvant therapy with S-1.

Results

Of the 64 patients included in the study, 6 refused adjuvant therapy with S-1 due to age (n=4) or financial concerns (n=2). The remaining 58 patients received S-1 within 8 weeks of surgery (Fig. 1). Twenty-four patients (41.3%) discontinued treatment within 12 months as a result of disease relapse (n=8) and intolerable adverse events (n=16). The S-1 dose was decreased in 9 of the 58 patients (15.5%). Of the 34 patients who underwent treatment for 12 months, the S-1 dose was decreased in 6 (17.6%), and of the 24 patients who discontinued treatment, the S-1 dose was decreased in 3 (12.5%). Among the 58 patients who received S-1 therapy, treatment was continued for at least 3 months in 49 patients (84.5%), at least 6 months in 45 patients (77.6%), at least 9 months in 37 patients (63.8%) and 12 months in 34 patients (58.6%).

Demographic and clinicopathological data of patients are shown in Table I. Patients who discontinued S-1 treatment within 12 months were older than those who completed 12 months of adjuvant therapy. However, no differences were observed in tumor stage and surgery type (gastrectomy, reconstruction or resection of other organs) between the two groups. Patients who completed 12 months of adjuvant therapy with S-1 were more frequently treated by senior doctors (>15 years of experience). More favorable outcomes in OS and relapse-free survival were observed in these patients than in those who discontinued treatment (Fig. 2).

Table II summarizes the data concerning the adverse reactions observed among the 58 patients in this study. No patient had ≥ grade 4 adverse events; however, 3 patients had grade 3 leukopenia. In terms of non-hematological adverse events, grade 3 anorexia was observed in 1 patient and grade 3 diarrhea was observed in 1 patient. The most frequent cause of S-1 treatment discontinuation was tumor recurrence. Non-hematological adverse events such as diarrhea and nausea were also associated with treatment discontinuation (Table III). Fig. 3 shows the medication completion rates. S-1 treatment time was significantly shorter in patients who

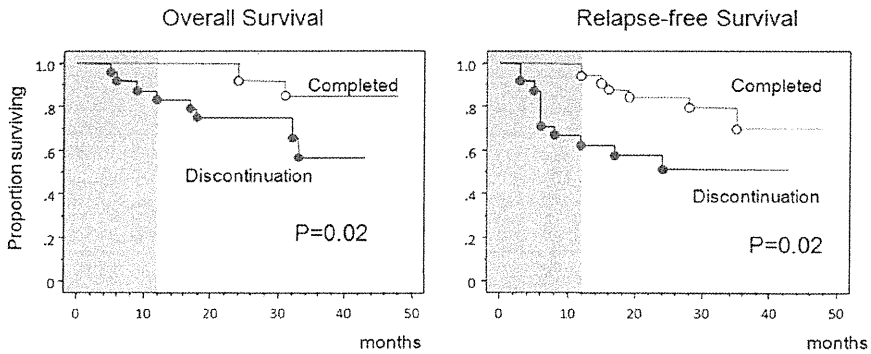

Figure 2. Overall and relapse-free survival rates following curative D2 gastric dissection. Completed, patients who completed 12 months of adjuvant therapy with S-1; Discontinuation, patients who discontinued treatment before 12 months.

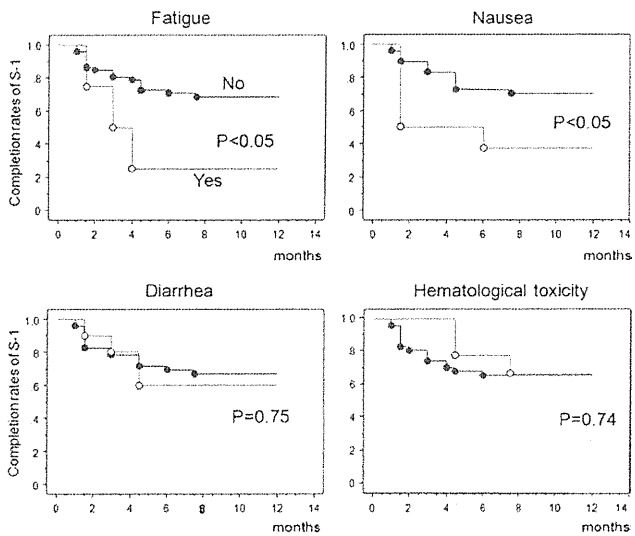

Figure 3. Medication completion rates of adjuvant therapy with S-1 calculated by the Kaplan-Meier method. Discontinuation of S-1 administration due to adverse events was considered as an event.

Table III. Chief causes of S-1 treatment discontinuation.

Adverse reaction	No. of patients	Percentage (%)
Recurrence	8	33.3
Diarrhea	3	12.5
Nausea	3	12.5
Elevated t-bil level	2	8.3
Intestinal obstruction	2	8.3
Fatigue	2	8.3
Neutropenia	1	4.2
Ascites	1	4.2
Stroke	1	4.2
Death of another cause	1	4.2
Total	24	100.0

t-bil, total bilirubin.

experienced fatigue or nausea as an adverse event, whereas diarrhea and hematological toxicity did not significantly affect the period of treatment.

Discussion

This study demonstrated that patients who completed 12 months of adjuvant therapy with S-1 were younger and more frequently treated by doctors with >15 years of experience than those who did not. Non-hematological adverse events such as nausea and fatigue were frequent causes of S-1 treatment discontinuation. Adverse events of ≥ grade 3 were significant causes of treatment discontinuation in a small number of patients.

In a postmarketing survey of S-1 (9), including 3,294 patients with advanced or recurrent gastric cancer, the incidence of adverse reactions following administration of the drug at the usual dose level according to the patient body surface area was 74.1%, which was approximately equal to that obtained in premarketing trials. The major reasons for drug discontinuation during the first and second course of therapy were exacerbation of symptoms (43%) and adverse drug reactions (33%). Therefore, to facilitate S-1 administration for prolonged time periods, the incidence of adverse reactions should be reduced. To accomplish this goal, several regimens have been established (10). Kimura *et al* developed a new S-1 dosing regimen in which S-1 is administered for a 2-week period separated by 1-week drug-free intervals, as adverse reactions due to S-1 therapy begin to appear 2-3 weeks after initial dosing (11). Sakuma *et al* also proposed alternate-day treatment with S-1 as a strategy for reducing toxicity, although the total dose of this regimen was 75% that of standard treatment (12). Both regimens decreased the incidence of adverse reactions and improved treatment compliance when compared with the conventional 4-week administration followed by a 2-week rest regimen.

In the ACTS-GC trial, 143 of 517 (27.7%) patients discontinued S-1 treatment due to adverse events, which was consistent with our results (27.6%). Only 5% patients in the ACTS-GC trial had metastasis or relapse of gastric cancer. Our study, which includes potentially more cases of advanced

stage disease than the ACTS-GC trial, involved relatively shorter time periods of the treatment than the ACTS-GC trial.

Patients who experienced fatigue or nausea as adverse events continued S-1 treatment for significantly shorter time periods. However, diarrhea and hematological toxicity did not significantly affect the treatment period. Following gastrectomy, fatigue and gastrointestinal symptoms such as nausea and appetite loss, even of ≤ grade 2, appeared to have a major impact on treatment compliance.

In conclusion, the completion rate of S-1 treatment did not depend on the type of surgical procedures, i.e., gastrectomy, reconstruction or resection of other organs. Fatigue and gastrointestinal symptoms affected the period of treatment continuation. In addition, patients who completed 12 months of adjuvant therapy with S-1 were more frequently treated by doctors with ≥15 years of experience. Thus, to facilitate the continuation of adjuvant therapy with S-1, patients and doctors must be made completely aware of the issues of toxicity, compliance and efficacy associated with this therapy.

References

1. Shirasaka T, Nakano K, Takechi T, Satake H, Uchida J, Fujioka A, *et al*: Antitumor activity of 1 M tegafur-0.4 M 5-chloro-2,4-dihydroxypyridine-1 M potassium oxonate (S-1) against human colon carcinoma orthotopically implanted into nude rats. Cancer Res 56: 2602-2606, 1996.
2. Tatsumi K, Fukushima M, Shirasaka T and Fujii S: Inhibitory effects of pyrimidine, barbituric acid and pyridine derivatives on 5-fluorouracil degradation in rat liver extracts. Jpn J Cancer Res 78: 748-755, 1987.
3. Shirasaka T, Shimamoto Y and Fukushima M: Inhibition by oxonic acid of gastrointestinal toxicity of 5-fluorouracil without loss of its antitumor activity in rats. Cancer Res 53: 4004-4009, 1993.
4. Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y and Taguchi T: Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur-0.4 M gimestat-1 M otastat potassium) in advanced gastric cancer patients. Eur J Cancer 34: 1715-1720, 1998.
5. Koizumi W, Kurihara M, Nakano S and Hasegawa K: Phase II study of S-1, a novel oral derivative of 5-fluorouracil, in advanced gastric cancer. For the S-1 Cooperative Gastric Cancer Study Group. Oncology 58: 191-197, 2000.
6. Japanese Gastric Cancer Association: Japanese Classification of Gastric Carcinoma - 2nd English Edition. Gastric Cancer 1: 10-24, 1998.
7. Sakuramoto S, Sasako M, Yamaguchi T, Kinoshita T, Fujii M, Nashimoto A, *et al*: Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med 357: 1810-1820, 2007.
8. Japanese Gastric Cancer Association: Japanese gastric cancer treatment guidelines 2010 (ver. 3). Gastric Cancer 14: 113-123, 2011.
9. Kawai H, Ohtsu A, Boku N, Hamamoto Y, Nagashima F, Muto M, *et al*: Efficacy and safety profile of S-1 in patients with metastatic gastric cancer in clinical practice: results from a post-marketing survey. Gastric Cancer 6: 19-23, 2003.
10. Iwasa S, Yamada Y, Fukagawa T, Eguchi Nakajima T, Kato K, Hamaguchi T, *et al*: Management of adjuvant S-1 therapy after curative resection of gastric cancer: dose reduction and treatment schedule modification. Gastric Cancer 14: 28-34, 2011.
11. Kimura Y, Kikkawa N, Iijima S, Kato T, Naoi Y, Hayashi T, *et al*: A new regimen for S-1 therapy aiming at adverse reaction mitigation and prolonged medication by introducing a 1-week drug-free interval after each 2-week dosing session: efficacy and feasibility in clinical practice. Gastric Cancer 6: 34-39, 2003.
12. Sakuma K, Hosoya Y, Arai W, Haruta H, Ui T, Kurashina K, *et al*: Alternate-day treatment with S-1 in patients with gastric cancer: a retrospective study of strategies for reducing toxicity. Int J Clin Oncol 15: 166-171, 2010.

Membranous and cytoplasmic expression of epidermal growth factor receptor in metastatic pancreatic ductal adenocarcinoma

TAKAHIRO EINAMA[1,6], SHIGETO UEDA[2], HITOSHI TSUDA[3,7], KAZUHIRO OGASAWARA[5], KAZUO HATSUSE[4], OSAMU MATSUBARA[3], SATORU TODO[1] and JUNJI YAMAMOTO[4]

[1]Department of General Surgery, Hokkaido University, Graduate School of Medicine, Sapporo; [2]Medical Service School, National Defense Force, Tokyo; Departments of [3]Basic Pathology and [4]Surgery, National Defense Medical College, Saitama; [5]Department of Surgery, Japan Labor Health and Welfare Organization Kushiro Rosai Hospital, Kushiro, Hokkaido; [6]Division of Gastroenterological and General Surgery, Department of Surgery, Asahikawa Medical University, Hokkaido, Japan

Received January 13, 2012; Accepted February 27, 2012

DOI: 10.3892/etm.2012.518

Abstract. Recent studies indicate the clinical significance of the cellular localization of epidermal growth factor receptor (EGFR) in a variety of cancer types. Internalization of activated EGFR is reported to be closely associated with patient prognosis. This study investigated the clinical significance of the immunohistochemical localization of EGFR in patients with metastatic pancreatic cancers compared to those with surgically resected pancreatic cancers. Using 44 surgically resected primary pancreatic cancers and 40 primary or metastatic tumors from 20 autopsied patients with far advanced pancreatic cancers, the incidence of membranous and cytoplasmic EGFR overexpression was compared between primary tumors and far advanced tumors by immunohistochemistry using the Dako EGFR pharmDx™ kit, a global standard kit for EGFR assay. In the 44 surgically resected cancers, 13 (30%) exhibited membranous overexpression of EGFR, comprising 1 case (2%) with score 3+ and 12 cases (27%) with score 2+ and 10 (23%) exhibited cytoplasmic overexpression of EGFR. In the 40 tumors at a far advanced stage, the percentage of samples exhibiting positivity for membranous and cytoplasmic EGFR overexpression was 48% (19 of 40) comprising 7 (18%) with score 2+ and 12 (30%) with score 3+ and 33% (13 of 40), respectively. The far advanced tumors tended to show membranous and cytoplasmic EGFR overexpression more frequently than the surgically resected tumors, although the difference was not significant. These findings suggest that membranous and cytoplasmic overexpression of EGFR may be indicative of the potential aggressiveness of pancreatic cancers.

Correspondence to: Dr Hitoshi Tsuda, [7]*Present address:* Pathology Section, Clinical Laboratory Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
E-mail: hstsuda@ncc.go.jp

Key words: epidermal growth factor receptor, metastatic pancreatic cancer, cytoplasmic expression

Introduction

Despite recent advances in diagnostic and therapeutic techniques, pancreatic carcinoma is one of the most lethal malignancies among cancers. The 5-year survival rate of patients having primary pancreatic cancer after complete resection does not reach 15% (1), while the overall 5-year survival rates in patients having inoperable pancreatic cancer are desperately low, ranging from 0.4 to 4% (2,3).

Currently, gemcitabine is a key drug not only for treating advanced pancreatic cancer (4) but also as an adjuvant chemotherapy regimen for resectable pancreatic cancer (5,6). Furthermore, molecular targeting of epidermal growth factor receptor (EGFR) or vascular endothelial growth factor (VEGF) has recently been developed to treat these lesions (7,8). Moore *et al* reported in a phase III trial of patients with advanced pancreatic cancer, that erlotinib, a tyrosine kinase inhibitor of EGFR, in combination with gemcitabine was superior to gemcitabine alone when progression-free and overall survival were compared between the two groups (8).

EGFR, one of the tyrosine kinase receptors of the ErbB family, is reported to be expressed immunohistochemically in 10-30% of patients with solid tumors including pancreatic carcinoma (9,10). Tyrosine phosphorylation in EGFR protein in cancer cells leads to activation of several downstream intracellular substrates and plays a pivotal role in tumor proliferation, invasion and metastasis (11). Recent studies have suggested that the EGFR gene copy number and expression obtained by fluorescence *in situ* hybridization (FISH) and immunohistochemistry (IHC) predict the clinical response of a tumor to gefitinib, a tyrosine kinase inhibitor of EGFR, in patients with non-small cell lung cancer (12-14). Furthermore, recent studies have found that mutations of the EGFR gene at the restricted region, e.g., exon 19 and exon 21, were closely correlated with response to gefitinib therapy (15-20). However, the relevance of EGFR expression in pancreatic cancer with therapeutic response has remained to be verified (8).

Although immunohistochemical expression of EGFR can also be recognized as positive membranous staining,

Table I. Clinicopathological characteristics of the patients and tumors.

	Surgically resected cancers (n=44)	Far advanced cancers (n=20)	
Parameter	n (%)	n (%)	
Age (mean ± SD, years)	63.3±3.7	57.3±5.7	
<65	23 (52)	13 (65)	
≥65	21 (47)	7 (35)	
Gender			
Male	34 (77)	16 (80)	
Female	10 (23)	4 (20)	
Tumor site			
Head	36 (82)	12 (60)	
Body and/or tail	8 (18)	8 (40)	
Stage			
I	1 (2)		
II	32 (73)		
III	8 (18)		
IV	3 (7)		
Grade			
1	12 (27)	5 (25)[a]	2 (10)[b]
2	28 (64)	3 (15)[a]	9 (45)[b]
3	4 (9)	12 (60)[a]	9 (45)[b]
Median survival (mean ± SD, month)	24.5±10.3		

[a]Primary cancers. [b]Hepatic metastases. SD, standard deviation.

cytoplasmic expression of EGFR can frequently be observed in cancer cells of the pancreas. We previously reported that high cytoplasmic expression of EGFR in primary pancreatic cancer was significantly correlated with higher histological grade and poorer survival (10), suggesting that cytoplasmic EGFR expression could indicate a potentially aggressive or metastatic feature of pancreatic cancer. However, it is unclear whether localization of EGFR expression differs between primary and metastatic sites of pancreatic cancers at surgically resectable stages and those at inoperable far advanced stages.

The present study compared immunohistochemically the levels and localization of EGFR expression between surgically resected primary pancreatic cancers and far advanced cancers obtained at autopsy, in order to clarify the clinical impact of membranous and cytoplasmic EGFR overexpressions in far advanced pancreatic cancers.

Materials and methods

Patients and tumor specimens. This study was performed with approval by the Internal Review Board on Ethical Issues of the National Defense Medical College, Japan. The subjects of this study were 44 patients who underwent surgery with curative intent for primary pancreatic cancers between 1987 and 2000 at the National Defense Medical College Hospital, Tokorozawa, Japan. The clinicopathological characteristics of these cases are summarized in Table I.

The mean patient age was 63.3 years [±3.7 standard deviation (SD)]. Thirty-four (77.3%) were men and 10 (22.7%) were women. More than 80% of tumors were located in the head of the pancreas. As for stage, approximately 90% of the patients were assigned to stage II or stage III (21). Histologically, all 44 patients had invasive ductal adenocarcinoma of the pancreas, and the majority of the patients had moderately differentiated tubular adenocarcinoma. The median survival time was 24.5 months (±10.3 SD).

In addition, a total of 40 tumor specimens from primary sites and hepatic metastatic sites were obtained at autopsy from 20 patients who had died of inoperable far advanced pancreatic cancer between 1980 and 2001 at the same hospital (Table I).

Using these tumor specimens from a total of 64 patients, formalin-fixed paraffin-embedded tissue blocks were prepared, and sections were cut and stained with hematoxylin and eosin (H&E) for routine histopathological examination. Because surgically resected specimens had been cut routinely for pathology specimens once a weak periodically, the duration of formalin fixation of the surgically resected specimens varied from 1 to 6 days. Likewise, the duration of formalin fixation of the autopsied tissues varied from 1 to 6 days. All specimens were diagnosed as ductal adenocarcinomas of the pancreas. After a histological review of the sections by three observers (T.E., H.T. and S.U.), a representative tissue block was selected from each surgically resected primary tumor, each primary tumor obtained by autopsy, and each metastatic tumor obtained

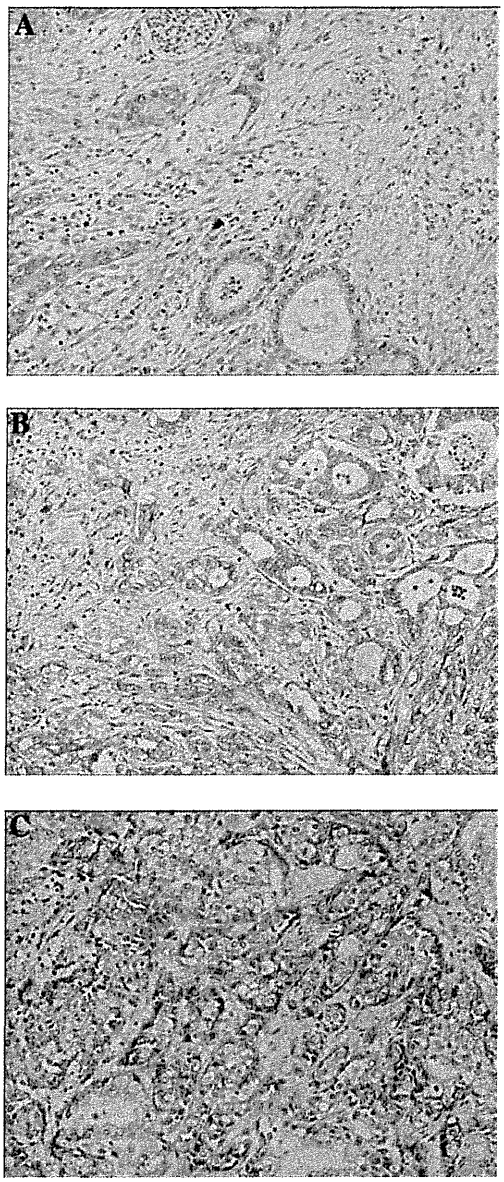

Figure 1. Representative cases of pancreatic ductal adenocarcinoma showing scores of 1+, 2+ and 3+ for membranous EGFR expression. (A) Score 1+, incomplete membrane staining is weakly visible. (B) Score 2+, the entire circumference of the cell membrane is weakly stained. (C) Score 3+, the entire circumference of the cell membrane is heavily stained. Immunoperoxidase stain, x200.

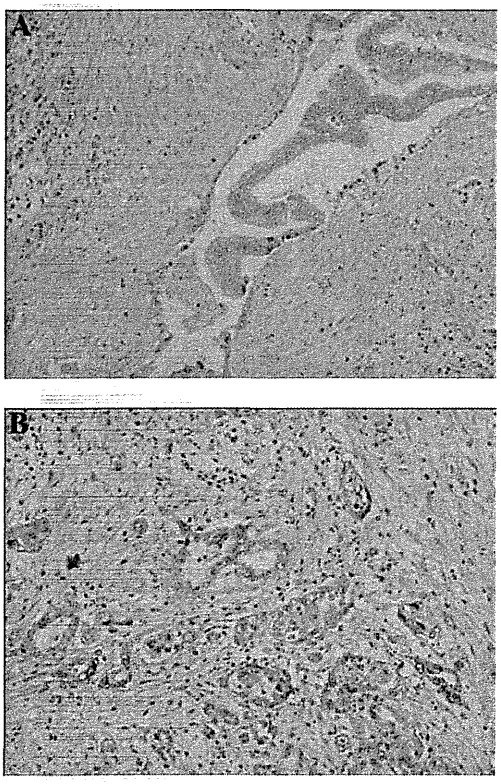

Figure 2. Representative cases of pancreatic ductal adenocarcinoma showing scores of 1+ and 2+ for EGFR cytoplasmic expression. (A) Score 1+, faint diffuse cytoplasmic staining is detected. (B) Score 2+, moderate to strong cytoplasmic staining and strong granular staining is observed. Immunoperoxidase stain, x200.

by autopsy. These tumor tissue blocks were subjected to immunohistochemical studies.

Histological classification. The three observers graded the degree of tumor differentiation. Tumor differentiation was classified into Grade 1 (well-differentiated type), Grade 2 (moderately differentiated type) and Grade 3 (poorly differentiated type), according to the degree of tubular formation (21). The grade of each primary cancer was defined according to the findings in the widest area of the representative section of the cancer.

Immunohistochemistry. Immunohistochemical staining for EGFR was performed using the EGFR pharmDx™ kit (Dako, Carpinteria, CA, USA), a global standard kit for EGFR assay approved by the US Food and Drug Administration (US FDA). Sections were deparaffinized in two sequential xylene baths (5 min), 100% ethanol (3 min) and 95% ethanol (3 min), followed by a 5-min single wash in wash-buffer solution (Dako). Subsequently, at room temperature, the section was rinsed in wash-buffer for 5 min, incubated in proteinase K solution (Dako) for 5 min, rinsed again in the wash-buffer for 5 min, incubated in peroxidase blocking agent for 5 min, rinsed, incubated with the primary EGFR antibody or negative control reagent for 30 min, rinsed, incubated with visualization reagent for 30 min, rinsed twice with the buffer, incubated with substrate chromogen solution for 5 min and finally rinsed again with the buffer. Slides were counterstained with hematoxylin and rinsed gently in reagent quality water. The positive and negative controls used were formalin-fixed, paraffin-embedded pellets of HT-29 and CAMA-1 cell lines, which expressed and did not express EGFR, respectively (Dako).

Immunohistochemical evaluation. Immunohistochemical evaluation was performed for both the cell membrane and cytoplasm, separately, for the primary or metastatic carcinoma samples. The level of membranous EGFR expression was stratified into 4 groups (scores 0, 1+, 2+ and 3+) according to the criteria for the HER2 test (HercepTest) (22). In detail, when membranous staining was observed in <10% of the tumor

Table II. EGFR immunostaining in the surgically resected cancers and the far advanced cancers obtained at autopsy.

	No. of cases (%)							
		Membranous EGFR reactivity				Cytoplasmic EGFR reactivity		
	Total	0	1+	2+	3+	0	1+	2+
Surgically resected cancers	44	24 (55)	7 (16)	12 (27)	1 (2)	22 (50)	12 (27)	10 (23)
Far advanced cancers	40	6 (15)	15 (38)	7 (18)	12 (30)	4 (10)	23 (58)	13 (33)
Primary cancers[a]	20	3 (15)	9 (45)	3 (15)	5 (25)	2 (10)	13 (65)	5 (25)
Hepatic metastases[a]	20	3 (15)	6 (30)	4 (20)	7 (35)	2 (10)	10 (50)	8 (40)

[a]No significant difference between membranous and cytoplasmic EGFR reactivity.

Table III. Expression of EGFR stratified according to histological grading between the surgically resected cancers and the far advanced cancers obtained at autopsy.

		No. of tumors (%)			
Histological grade	Total	Membranous EGFR overexpression	P-value[a]	Cytoplasmic EGFR overexpression	P-value[a]
Surgically resected cancers	44	13 (30)[b]		10 (23)[c]	
Grade 1	12	1 (8)	0.07	1 (8)	0.2
Grade 2/3	32	12 (38)		9 (28)	
Far advanced cancers	40	19 (48)[b]		13 (33)[c]	
Grade 1	7	3 (43)	0.8	1 (14)	0.3
Grade 2/3	33	16 (48)		12 (36)	

[a]P-value indicates comparisons between Grade 1 and Grade 2/3 tumors. No significant difference was noted between EGFR expression and histological grade (Grade 1 vs. 2/3). [b]P=0.09, statistically significant difference between surgically resected and advanced cancers. [c]P=0.3, statistically significant difference between surgically resected and advanced cancers.

cells, a score of 0 was assigned, regardless of the intensity of the staining. If faint or barely perceptible membranous staining was detected in >10% of the tumor cells, a score of 1+ was assigned. Scores of 2+ and 3+ were assigned when weak to moderate staining and strong staining, respectively, were observed on the entire membrane in >10% of the tumor cells (Fig. 1). Cases showing a score of 2+ or 3+ were defined as showing overexpression.

Cytoplasmic staining was divided into 3 grades (0, 1+ and 2+), as grading of the intensity of the immunoreaction was difficult for the cytoplasm. The level of cytoplasmic staining was categorized as follows: when cytoplasmic staining was observed in <10% of the tumor cells, a score of 0 was assigned. If faint or barely perceptible cytoplasmic staining was detected in >10% of tumor cells, a score of 1+ was assigned. A score of 2+ was assigned when moderate or strong staining, respectively, was observed in >10% of the tumor cells. Cytoplasmic granular staining was also scored as 2+. Cases showing a score of 2+ were judged as showing overexpression (Fig. 2).

Statistical analysis. We used the χ^2 test or Fisher's exact test to determine the correlation between EGFR expression and histological grade. Differences were considered to indicate statistical significance at a P-value <0.05. All statistical analyses were performed using Statview 5.0 software (SAS Institute Inc., Cary, NC, USA).

Results

The expression profiles of membranous and cytoplasmic EGFR in both surgically resected cancers and far advanced cancers obtained at autopsy are shown in Table II. In the 44 surgically resected cancers, 13 (30%) exhibited membranous overexpression of EGFR, comprising 1 case (2%) of score 3+ and 12 cases (27%) of score 2+ and 10 (23%) exhibited cytoplasmic overexpression of EGFR.

In the primary tumors in the 20 far advanced cancers, the percentage of samples with positivity for membranous EGFR overexpression was 40%, (8 of 20), comprising 3 cases (15%) of score 2+ and 5 cases (25%) of score 3+, and the percentage of samples showing positivity for cytoplasmic EGFR overexpresion was 25% (5 of 20). In the hepatic metastases in the 20 far advanced cancers, the positivity of membrane EGFR overexpression was 55%, (11 of 20), comprising 4 cases (20%) of score 2+ and 7 cases (35%) of score 3+, and the positivity of cytoplasmic EGFR overexpresion was 40% (8 of 20).

In a total of 40 tumors at a far advanced stage, the percentage of samples showing positivity for membranous EGFR overexpression was 48% (19 of 40) comprising 7 cases (18%) of score 2+ and 12 cases (30%) of score 3+, and the percentage of samples showing positivity for cytoplasmic EGFR overexpresion was 33% (13 of 40). Therefore, the far advanced tumors tended to show membranous and cytoplasmic EGFR overexpression more frequently than the surgically resected tumors, although the difference was not significant.

When these cases were stratified according to histological grade, higher grade (Grades 2 and 3) cancer tissues tended to show membranous EGFR overexpression more frequently (12 of 32, 38%) than the lower grade (Grade 1) cancer tissues (1 of 12, 8%) in the surgically resected pancreatic cancers, although the difference was statistically marginal (P=0.07). The percentage of cytoplasmic EGFR overexpression did not differ statistically between the low grade (Grade 1) tumors (1 of 12, 8%) and higher grade (Grades 2 and 3) tumors (9 of 32, 28%) in the surgically resected cases.

The tissues of the far advanced cancers showed similar rates of membranous and cytoplasmic overexpressions, regardless of histological grade. In the 40 far advanced tumors, membranous EGFR overexpression was detected in 3 (43%) of 7 Grade 1 cases and in 16 (48%) of Grade 2 or 3 cases. In these far advanced tumors, cytoplasmic overexpression of EGFR was detected in 14% (1 of 7) of Grade 1 tumors and 36% (12 of 33) of Grade 2 or 3 tumors (Table III).

Discussion

In the present study, we demonstrated that EGFR overexpression in the cell membrane and cytoplasm was common in both surgically resected and far advanced pancreatic carcinomas. The occurrences of membranous and cytoplasmic EGFR overexpression tended to be higher in the tumors at far advanced stages than in the tumors that were at surgically resectable stages as determined using a global standard kit for EGFR assay.

Cytoplasmic EGFR expression in the far advanced cancers may be explained by the hypothesis of epithelial-to-mesenchymal transition which is thought be an important mechanism for promoting cancer invasion and metastasis (23). Persistently activated EGFR can decrease intercellular adhesion between tumor cells and enhance cancer cell migration. Willmarth *et al* showed that EGF-activated EGFR in MCF10A cells enhanced signal transduction predominantly from the endosomes rather than from the membrane (24). Barr *et al* (25) suggested that continuously EGF-treated EGFR induced endocytosis of E-cadherin, a cell-to-cell adhesion protein, which enhanced invasiveness in several human cancer cell lines. Ueda *et al* previously reported that EGFR overexpression in the cytoplasm of pancreatic cancers was associated with poorer clinical outcome of patients (10,26). The present study corroborated that not only membranous overexpression but also cytoplasmic overexpression of EGFR is important for the acquisition of highly aggressive and metastatic properties of pancreatic carcinomas.

In the present study, the rate of EGFR overexpression in surgically resected cancers tended to be higher in higher grade (Grades 2 and 3) tumors than in low grade (Grade 1) tumors. It is not surprising that poorly differentiated pancreatic cancers exhibited a higher incidence of EGFR overexpression as the patients with pancreatic carcinoma with altered EGFR activity tend to show a more aggressive clinical course and a poorer clinical outcome (27). Aggressive tumors appear to require the activation of an EGFR-mediated autocrine signaling in order to maintain proliferation. Therefore, we suppose the possibility that cytoplasmic EGFR protein, which is newly synthesized within the endoplasm reticulum, would be processed at the cellular surface. Some investigators reported that binding of EGF to EGFR activates its receptor tyrosine kinases and accelerates its internalization through clathrin-coated pits followed by the efficient lysosomal targeting of internalized receptors, which results in receptor downregulation and degradation. Thus, the ligand-induced internalization of EGFR, so-called endocytosis trafficking, is characterized as activated EGFR (28-30). If the EGFR ligands dissociated EGFR localized in endosomes, EGFR would be deactivated and recycled to the plasma membrane.

We should consider the possibility that EGFR localization and its activity in advanced or metastatic pancreatic cancers may be modulated by chemotherapy or radiation therapy which those patients had received. It is known that ionizing radiation, hypoxia and oxidative stress can also phosphorylate EGFR with ligand independence, which is sequentially internalized and shuttled into the nucleus (31). Li *et al* (32) reported that the non-small cell lung cancer H226 cells which acquire resistance to cetuximab, an anti-EGFR antibody, showed decreased membranous EGFR accompanied by EGFR expressed with nuclear localization. These findings imply that EGFR localization of cancer cells may be an important determinant of responsiveness to specific therapies.

In conclusion, we demonstrated using immunohistochemistry that membranous and cytoplasmic EGFR overexpression was frequently noted in surgically resected and far advanced pancreatic cancers. These findings suggest that membranous and cytoplasmic overexpression of EGFR may be indicative of the potential aggressiveness of pancreatic cancers.

Acknowledgements

This study was supported in part by a grant-in-aid for defense medicine from the Ministry of Defense and a grant-in-aid from the Foundation for Promotion of Defense Medicine. The authors thank Dr Tatsuro Takahashi and Mr. Ryuji Saito, Department of Central Laboratory, Japan Labor Health and Welfare Organization Kushiro Rosai Hospital, Kushiro, Hokkaido, Japan, for technical assistance with the immunohistochemistry.

References

1. Matsuno S, Egawa S, Fukuyama S, *et al*: Pancreatic Cancer Registry in Japan: 20 years of experience. Pancreas 28: 219-230, 2004.
2. Bramhall SR, Allum WH, Jones AG, Allwood A, Cummins C and Neoptolemos JP: Treatment and survival in 13,560 patients with pancreatic cancer, and incidence of the disease, in the West Midlands: an epidemiological study. Br J Surg 82: 111-115, 1995.
3. Parkin DM, Bray FI and Devesa SS: Cancer burden in the year 2000. The global picture. Eur J Cancer 37 (Suppl 8): S4-S66, 2001.

4. Burris HA III, Moore MJ, Andersen J, *et al*: Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 15: 2403-2413, 1997.
5. Oettle H, Post S, Neuhaus P, *et al*: Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA 297: 267-277, 2007.
6. Ueno H, Kosuge T, Matsuyama Y, *et al*: A randomised phase III trial comparing gemcitabine with surgery-only in patients with resected pancreatic cancer: Japanese Study Group of Adjuvant Therapy for Pancreatic Cancer. Br J Cancer 101: 908-915, 2009.
7. Kindler HL, Friberg G, Singh DA, *et al*: Phase II trial of bevacizumab plus gemcitabine in patients with advanced pancreatic cancer. J Clin Oncol 23: 8033-8040, 2005.
8. Moore MJ, Goldstein D, Hamm J, *et al*: Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 25: 1960-1966, 2007.
9. Salomon DS, Brandt R, Ciardiello F and Normanno N: Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 19: 183-232, 1995.
10. Ueda S, Ogata S, Tsuda H, *et al*: The correlation between cytoplasmic overexpression of epidermal growth factor receptor and tumor aggressiveness: poor prognosis in patients with pancreatic ductal adenocarcinoma. Pancreas 29: e1-e8, 2004.
11. Mendelsohn J and Baselga J: Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. J Clin Oncol 21: 2787-2799, 2003.
12. Cappuzzo F, Hirsch FR, Rossi E, *et al*: Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small cell lung cancer. J Natl Cancer Inst 97: 643-655, 2005.
13. Hirsch FR, Varella-Garcia M, McCoy J, *et al*: Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. J Clin Oncol 23: 6838-6845, 2005.
14. Tsao MS, Sakurada A, Cutz JC, *et al*: Erlotinib in lung cancer – molecular and clinical predictors of outcome. N Engl J Med 353: 133-144, 2005.
15. Lynch TJ, Bell DW, Sordella R, *et al*: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small cell lung cancer to gefitinib. N Engl J Med 350: 2129-2139, 2004.
16. Han SW, Kim TY, Hwang PG, *et al*: Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small cell lung cancer patients treated with gefitinib. J Clin Oncol 23: 2493-2501, 2005.
17. Mitsudomi T, Kosaka T, Endoh H, *et al*: Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small cell lung cancer with postoperative recurrence. J Clin Oncol 23: 2513-2520, 2005.
18. Paez JG, Janne PA, Lee JC, *et al*: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 304: 1497-1500, 2004.
19. Takano T, Ohe Y, Sakamoto H, *et al*: Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small cell lung cancer. J Clin Oncol 23: 6829-6837, 2005.
20. Takano T, Ohe Y, Tsuta K, *et al*: Epidermal growth factor receptor mutation detection using high-resolution melting analysis predicts outcomes in patients with advanced non-small cell lung cancer treated with gefitinib. Clin Cancer Res 13: 5385-5390, 2007.
21. Sobin LH and Wittekind Ch (eds): International Union Against Cancer (UICC). TNM Classification of Malignant Tumors. 6th edition. Wiley-Liss, New York, 2002.
22. Tsuda H, Akiyama F, Terasaki H, *et al*: Detection of HER-2/neu (c-erb B-2) DNA amplification in primary breast carcinoma. Interobserver reproducibility and correlation with immunohistochemical HER-2 overexpression. Cancer 92: 2965-2974, 2001.
23. Dembinski JL and Krauss S: Characterization and functional analysis of a slow cycling stem cell-like subpopulation in pancreas adenocarcinoma. Clin Exp Metastasis 26: 611-623, 2009.
24. Willmarth NE, Baillo A, Dziubinski ML, Wilson K, Riese DJ II and Ethier SP: Altered EGFR localization and degradation in human breast cancer cells with an amphiregulin/EGFR autocrine loop. Cell Signal 21: 212-219, 2009.
25. Barr S, Thomson S, Buck E, *et al*: Bypassing cellular EGF receptor dependence through epithelial-to-mesenchymal-like transitions. Clin Exp Metastasis 25: 685-693, 2008.
26. Ueda S, Hatsuse K, Tsuda H, *et al*: Potential crosstalk between insulin-like growth factor receptor type 1 and epidermal growth factor receptor in progression and metastasis of pancreatic cancer. Mod Pathol 19: 788-796, 2006.
27. Holbro T, Civenni G and Hynes NE: The ErbB receptors and their role in cancer progression. Exp Cell Res 284: 99-110, 2003.
28. Madshus IH and Stang E: Internalization and intracellular sorting of the EGF receptor: a model for understanding the mechanisms of receptor trafficking. J Cell Sci 122: 3433-3439, 2009.
29. Sorkin A and Goh LK: Endocytosis and intracellular trafficking of ErbBs. Exp Cell Res 315: 683-696, 2009.
30. Sorkin A and von Zastrow M: Endocytosis and signalling: intertwining molecular networks. Nat Rev Mol Cell Biol 10: 609-622, 2009.
31. Dittmann K, Mayer C, Kehlbach R and Rodemann HP: Radiation-induced caveolin-1 associated EGFR internalization is linked with nuclear EGFR transport and activation of DNA-PK. Mol Cancer 7: 69, 2008.
32. Li C, Iida M, Dunn EF, *et al*: Nuclear EGFR contributes to acquired resistance to cetuximab. Oncogene 28: 3801-3813, 2009.

World J Surg (2012) 36:2187–2191
DOI 10.1007/s00268-012-1622-0

World Journal of Surgery

The Implications of Positive Peritoneal Lavage Cytology in Potentially Resectable Pancreatic Cancer

Ryuji Yoshioka · Akio Saiura · Rintaro Koga · Junichi Arita · Nobuyuki Takemura · Yoshihiro Ono · Junji Yamamoto · Toshiharu Yamaguchi

Published online: 4 May 2012

Abstract
Background The clinical implications of peritoneal lavage cytology (CY) status in patients with potentially resectable pancreatic cancer have not been established.
Method We retrospectively reviewed clinical data from 254 consecutive patients who underwent macroscopically curative resection for pancreatic cancer from February 2003 to December 2010 in our institution. Correlations between CY status and survival and clinicopathological findings were investigated.
Results Of the 254 patients, 20 were CY+ (7.9 %). There were no significant differences between CY+ and CY− patients in background data (age, sex, the level of preoperative tumor marker, and adjuvant chemotherapy). Patients with positive serosal invasion were more likely to be CY+ than those with negative serosal invasion ($P < 0.001$) by univariate analysis. The median overall survival of CY+ patients and CY− patients was 23.8 months (95 % CI = 17.6–29.8) and 26.5 months (95 % CI = 20.7–32.3), respectively ($P = 0.302$). The median recurrence-free survival of CY+ and CY− patients was 8.1 months (95 % CI = 0.0–17.9) and 13.5 months (95 % CI = 11.5–15.5), respectively ($P = 0.089$).
Conclusion CY+ status without other distant metastasis does not necessarily preclude resection in patients with pancreatic cancer.

Presented at the European Society for Medical Oncology 13th World Congress on Gastrointestinal Cancer, Barcelona, Spain, June 2011.

R. Yoshioka · A. Saiura (✉) · R. Koga · J. Arita · N. Takemura · Y. Ono · T. Yamaguchi
Department of Gastroenterological Surgery, Cancer Institute Hospital, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto-ku, Tokyo 135-8550, Japan
e-mail: saiura-tky@umin.ac.jp

J. Yamamoto
Department of Hepato-Biliary-Pancreatic Surgery, National Defense Medical College Hospital, Tokorozawa, Japan

Introduction

Pancreatic adenocarcinoma is the fourth leading cause of cancer-related death in the United States and is associated with an extremely poor prognosis [1]. Surgical resection offers the only chance for cure; however, only 10–20 % of patients are considered candidates for surgical resection. Patients with locally advanced/unresectable disease (30–40 %) or metastatic disease (50–60 %) are excluded from consideration for resection [2].

Peritoneal lavage cytology (CY) is used widely in the diagnosis and staging of ovarian and gastric cancer [3–5]. In pancreatic cancer, malignant cancer cells have been identified in 7–30 % of peritoneal washings [6–16], and The American Joint Committee on Cancer (AJCC) staging of pancreatic cancer includes positive CY findings (CY+) as indicative of stage IV disease [5]. Indeed, many studies have associated CY+ status with advanced disease and poor survival and concluded that CY+ status in potentially resectable pancreatic cancer should be considered a contraindication for radical surgery [6, 8, 10, 11, 14].

On the other hand, several authors reported that CY status is not always associated with poor prognosis in patients with potentially resectable pancreatic cancer, claiming that CY+ in the absence of macroscopic peritoneal metastasis or liver metastasis is not a contraindication for radical surgery [12, 13, 15, 16]. The clinical implications of CY status in potentially resectable pancreatic cancer without other distant metastasis thus remain controversial, and the present study sought to clarify this issue.

Patients and methods

Clinical data collected prospectively from 254 consecutive patients who underwent macroscopically curative resection for invasive ductal carcinoma of the pancreas in the absence of macroscopic liver or peritoneal metastasis from February 2003 to December 2010 in our institution were reviewed retrospectively. Correlations between CY status and survival and clinicopathological findings were investigated. Curative resection was defined as the macroscopic removal of all gross tumors without liver metastases or macroscopic peritoneal spreading.

Postoperative follow-up

Routine follow-up consisted of laboratory studies, including tumor markers at 3-month intervals and computed tomography imaging at 3–6-month intervals. Until March 2005, our institute participated in a multicenter randomized phase III trial comparing gemcitabine with surgery alone in patients with macroscopically resected pancreatic cancer [17]. After the study, gemcitabine-based adjuvant chemotherapy was routinely performed. Postoperative peritoneal recurrence was defined as the recurrence detected macroscopically by imaging.

Peritoneal lavage cytology

Peritoneal washing and cytological analysis were routinely performed at the time of surgical exploration using a normal saline introduced into the abdominal cavity. After gentle agitation, as much fluid as possible was collected by syringe and centrifuged. Cytological smears were prepared from the centrifuged deposit and examined by an experienced pathologist after Papanicolaou staining.

Statistical analysis

For univariate analysis, binomial variables were compared using Pearson's χ^2 test and Fisher's exact test. Continuous variables were compared using the Mann–Whitney U test. Only those variables with P values of 0.10 or less by univariate analysis were entered into multivariate analyses in a backward stepwise manner until all variables remaining in the model were significant. Survival curves were calculated using the Kaplan-Meier method and compared using log rank tests. P values less than 0.05 were considered significant. Statistical analyses were performed using SPSS v19.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results

There was no postoperative mortality. The patients were followed for a mean period of 24.7 months (range = 0.8–97.4 months). The conclusive stages, according to AJCC staging, of the 254 patients who underwent resection were IA in 10 patients, IB in 14 patients, IIA in 49 patients, IIB in 142 patients, III in 3 patients, and IV in 36 patients. Of the 254 patients, 20 were CY+ (7.9 %). Table 1 summarizes the patient demographics and clinical characteristics. There was no significant difference between the CY+ and CY− groups in age, sex, serum level of preoperative tumor markers, and the presence or absence of adjuvant chemotherapy. R1 resections were significantly more frequent in CY+ patients (10 of 20 patients; 50 %) than in CY− patients (58 of 234 patients; 24.8 %; Table 2).

The correlation between cytological status and clinicopathological parameters was analyzed (Table 3). Patients with positive serosal invasion were more likely to be CY+ than those with negative serosal invasion ($P < 0.001$). Multivariate analysis identified serosal invasion as the only independent factor associated with CY status ($P < 0.001$;

Table 1 Patient characteristics

Age (years) [mean (range)]	66.4 (42–89)
Gender	
Male	138
Female	116
Stage	
IA	10
IB	14
IIA	49
IIB	142
III	3
IV	36
Operation	
Pancreatoduodenectomy	172
Distal pancreatectomy	78[a]
Total pancreatectomy	3
Central pancreatectomy	1
CY	
Negative	234
Positive	20
R0/1	
R0	186
R1	68
Survival time (months) [median (95 % CI)]	24.7 (19.8–29.6)

CY+ positive peritoneal lavage cytology

[a] Including three patients of Appleby procedure

Table 2 Comparison of background data between CY+ and CY− patients

	CY−	CY+	*P*
Age (years) [mean (range)]	66.6 (42–87)	63.4 (42–89)	0.212
Gender			
Male	124	14	
Female	110	6	0.143
Preoperative tumor marker [mean (range)]			
CEA (ng/ml)	6.0 (0.0–145.4)	3.8 (0.9–17.0)	0.699
CA19-9 (U/ml)	1136.0 (0.1–50,000)	4786.1 (2.0–45,894.7)	0.151
CA125 (U/ml)	33.2 (4.5–1,108.0)	17.7 (6.5–37.8)	0.957
Adjuvant chemotherapy			
Yes	166	16	
No	68	4	0.388
R0/1			
R0	176	10	
R1	58	10	0.015*

CY+ positive peritoneal lavage cytology

* Statistically significant

Table 3 Univariate analysis of correlation between cytological status and pathological parameters

	CY−	CY+	*P* value
Differentiation			
Well/mod	187	17	
Other	47	3	0.773
Tumor size			
<2 cm	24	1	
>2 cm	240	19	0.703
Serosal invasion			
Negative	201	10	
Positive	33	10	<0.001*
Retroperitoneal invasion			
Negative	72	6	
Positive	162	14	0.943
Bile duct invasion			
Negative	140	14	
Positive	94	6	0.372
Duodenal invasion			
Negative	165	11	
Positive	69	9	0.118
Portal vein invasion			
Negative	182	12	
Positive	52	8	0.097
Arterial invasion			
Negative	230	19	
Positive	4	1	0.339
Perineural invasion			
Negative	159	15	
Positive	75	5	0.515
Lymph node metastasis			
Negative	72	3	
Positive	162	17	0.138

CY+ positive peritoneal lavage cytology

*Statistically significant

odds ratio [OR] = 6.091; 95 % confidence interval [CI] = 2.354–15.760).

The median overall survival of CY+ patients and CY− patients was 23.8 (95 % CI = 17.6–29.8) months and 26.5 (95 % CI = 20.7–32.3) months, respectively ($P = 0.302$), while the median recurrence-free survival (RFS) of CY+ and CY− patients was 8.1 (95 % CI = 0.0–17.9) months and 13.5 (95 % CI = 11.5–15.5) months, respectively ($P = 0.089$; Fig. 1).

Table 4 summarizes the distribution of initial recurrence site after resection. Peritoneal recurrence was more significantly frequent in CY+ patients (7 of 20 patients; 35 %) than in CY− patients (16 of 234 patients; 7 %).

Discussion

Several studies have evaluated the prognostic value of CY status in pancreatic cancer, with many associating CY+ status with advanced disease and poor prognosis. The National Comprehensive Cancer Network (NCCN) pancreatic adenocarcinoma guidelines state that "The panel considers positive cytology from washings obtained at laparoscopy or laparotomy to be equivalent to M1 disease" [18]. Many studies also concluded that CY+ status is a contraindication for resection [6–11, 14, 19]. Ferrone et al. [19] pointed out that CY+ patients who underwent resection without other distant metastasis had significantly worse survival than CY− patients and had a survival rate similar to that of patients with stage IV disease. On the other hand, Yachida et al. [15] suggested that this conclusion could be premature because only a small number of CY+ patients had undergone resection in the relevant studies. In addition, several studies found no significant correlation between CY status and survival in patients who had undergone potentially curative resection [12, 15, 16]. According to these three reports, the overall survival of CY− and CY+ patients who underwent pancreatic resection without liver metastasis and/or macroscopic peritoneal metastasis was not significantly different (Table 5), and the authors concluded that CY+ status without other distant metastasis is not a contraindication for radical surgery. The present study supports the latter results, and we therefore consider that the indication for resecting CY+ pancreatic