Clinicopathological Characteristics of Multiple Primary Cancers in Hepatobiliary and Pancreas Malignancies

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Abstract. *Aim: The present study was designed to define the clinicopathological characteristics of multiple cancers (MC) in 597 patients with hepatobiliary and pancreas (HBP) malignancies who underwent curative resection, in order to clarify risk factors and prognostic significance. Patients and Methods: Patients' demographics, clinicopathological parameters and survival rates were compared between solitary (SC) and MC HBP malignancies for 267 patients with hepatocellular carcinoma (HCC), 77 with intrahepatic cholangiocarcinoma (ICC), 84 with extrahepatic bile duct carcinoma (BDC), 72 with gallbladder carcinoma (GBC) and 97 with pancreatic cancer (PC). Results: MC was observed in 66 patients (11%) and more than three cancers were observed in 13 (2.2%). The mean age of patients of the MC group was significantly higher than that of the SC group. The proportion of Nagasaki atomic bomb survivors among the MC group was significantly higher than among the SC group. These findings were significant in HCC and ICC. The histopathological aggressiveness of malignancies was lower with HCC, BDC and PC. In HCC, the disease-free survival of MC patients with more than three tumors was significantly lower than those with SC and double cancers. In GBC, the overall survival of the MC group was significantly better than the SC group. In PC, the disease-free and overall survival were significantly better in MC than SC. Conclusion: Careful follow-up for second or third occurrence of primary malignancies after primary curative treatment for HBP malignancy is necessary.*

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Multiple cancers (MC), such as the hereditary non-polyposis colorectal cancer (HNPCC; Lynch's syndrome) and the multiple endocrine neoplasia type 1 or 2 are rare, and specific genetic or chromosomal aberrations are involved in their carcinogenesis and/or pathogenesis (1-3). On the other hand, the survival of patients with hepatobiliary-pancreas (HBP) malignancies has markedly improved (4-7). During follow-up after surgery, we sometimes experience the development of other primary malignancies, excluding recurrence of HBP. In sporadic primary cancers, the exact genetic aberration remains elusive and certain pathogenic factors, such as pre-existing chronic diseases, exposure to toxic substances and/or irradiation, are considered to play a role in the development of various malignancies (8-12). In today's clinical practice, it is not uncommon to diagnose the simultaneous presence of two or more cancers during a short period after surgery for HBP tumors. Based on this background, we hypothesized that the clinicopathological features and prognosis differ between patients with HBP malignancies who present with solitary tumors (SC) and those who are diagnosed with MC. To test our hypothesis, we examined patients' demographics, various clinicopathological factors and survival of 597 patients who underwent curative surgery for various HBP malignancies.

Patients and Methods

Patients. Information on 597 consecutive patients was retrieved from the database of Nagasaki University Graduate School of Biomedical Sciences (NUGBS). These patients represented all those who had undergone hepatic or pancreatic resection at the Division of Surgical Oncology and the Development of Surgery at NUGBS between 1994 and June 2013. The study protocols were approved by the Human Ethics Review Board of our institution. Informed consent for data collection was obtained from each patient before surgery

Table I. *Comparison between clinicopathological features and tumor recurrence in HCC patients with or without multiple cancers.*

	Single (n=244)	Multiple (n=23)	*p*	Double (n=16)	Multiple (>2) (n=7)
Age (years)	65±11	72±6	0.006	71±7	75±5
Gender (males/females)	190/54	21/2	0.181	15/1	6/1
History of atomic bomb (n=257) (no/yes)	208/36	6/7	0.002	6/1	0/6**
Occupation (n=74) (none or office worker / manufacturing / laborer)	51/0/0	21/1/1	0.102	16/0/0	5/1/1
Residential area (n=74) (city/ countryside)	41/10	21/2	0.320	16/0	5/2
Diabetes (n=74) (no/yes)	34/17	12/11	0.351	8/8	4/3
Smoking (n=74) (no/yes)	37/14	14/9	0.463	11/5	3/4
Background liver disease (normal/chronic hepatitis/cirrhosis)	8/143/93	1/15/7	0.757	0/9/7	1/6/0
Cause of liver injury (HBV/HCV/HB-HCV/alcoholic/NASH/none)	76/95/18/9/2/44	5/10/1/1/0/6	0.858	5/6/1/0/0/4	0/4/0/1/0/2
Child-Pugh classification (A/B)	215/29	21/2	0.712	15/1	6/1
Liver damage grade (A/B)	186/58	19/4	0.868	13/3	6/1
Pretreatment for HCC (no/yes)	177/67	20/3	0.212	15/1	5/2
Alpha feto-protein level (ng/ml)	4420±14101	6071±40701	0.780	2013±6388	8548±22142
PIVKA-II level (mAU/ml)	2400±5929	7398±25758	0.130	1076±2616	5048±9490
AFP-L3 fraction level (%) (n=126)	18±25	13±18	0.274	12±19	11±19
Macroscopic finding of HCC (SN/SNEG/CM or IF)	72 /90/82	10/12/1	0.015	7/9/0	3/3/1
Number of HCC	1.3±0.5	1.3±0.5	0.915	1.3±0.5	1.3±0.5
Size of tumor (cm)	52±42	33±15	0.009	31±14	38±16
Milan criteria (met/not-met)	142/102	17/6	0.213	12/4	5/2
Vascular infiltration (no/yes)	164/80	15/8	1.0	10/6	5/2
Degree of fibrosis (Staging) (n=209)	2.6±1.3	2.5±1.3	0.742	2.8±1.5	1.8±0.8
Degree of necro-inflammatory response (grading) (n=202)	1.7±1.4	2.3±1.6	0.494	2.6±1.8	1.8±1.2
Japan TNM stage I/II/III/IV-A/IV-B	33/95/67/45/4	5/9/5/3/1	0.626	5/5/4/2/0	0/4/1/1/1
Extent of hepatectomy (partial resection/segmentectomy/hemi-hepatectomy)	71/92/81	11/8/4	0.129	9/5/2	2/3/2
Postoperative complications					
Hepatic failure (no/ yes)	226/18	23/0	0.439	16/0	7/0
Uncontrolled ascites (no/yes)	184/60	20/3	0.507	14/2	6/1
Postoperative HCC recurrence (no/yes)	73/171	11/12	0.249	10/6	1/6

*Macroscopic findings by the General Rules for the Clinical and Pathological Study of Primary Liver Cancer in Japan (13). SN, Simple nodular; SNEG, simple nodular with extranodular growth; CM, confluent multinodular; IF, infiltrative(13), AFP, alpha feto-protein. PIVKA, protein induced by Vitamin K absence or antagonist. **$p<0.01$ *vs.* the double-cancer group.

and the agreement of retrospective survey was performed by public announcement. No financial support was received for this study and the authors declare no conflict of interest. In addition to the NUGBS database, we obtained information on patients' demographics, various preoperative clinical and pathological parameters, surgical records and prognosis from the medical charts where necessary. The clinicopathological parameters were followed by the General Rules for Clinical and Pathologic studies on Cancer of the Colon, Rectum and Anus(13), the Classification of Biliary Tract Carcinoma in Japan (14) and the Classification of Pancreatic Carcinoma(15).

Statistics. The Fisher's exact test and Dunnett's multiple comparison test were used for analysis of categorical data, while the Student's t-test was used for continuous variables with normal distributions. The disease-free survival from the date of diagnosis until the date of death was estimated according to the Kaplan-Meier method. Consistent discrepancies in mortality among each group were tested using the log-rank test. All statistical tests were two-sided and $p<0.05$ was considered statistically significant. The SPSS software package (version 18.0; IBM Company, Chicago, IL, USA) was used for the calculations.

Results

The study cohort included 413 males and 184 females with a mean age, at time of surgery, of 66.5±11.4 years (range=28-87 years). The reason for surgery was hepatocellular carcinoma (HCC; n=267, 45%), intrahepatic cholangiocarcinoma (ICC; n=77, 13%), extrahepatic bile duct carcinoma (BDC;

Table II. *Comparison between clinicopathological features and tumor recurrence in ICC patients with or without multiple cancers.*

	Solitary (n=61)	Multiple (n=16)#	*p*-Value
Age (years)	66±11	71±7	0.045
Gender	(males/females)	37/24	12/4
0.386			
Atomic bomb survivor (no/yes)	49/12	8/8	0.018
Occupation (none or office worker/manufacturing/ laborer)	46/9/6	10/4/2	0.558
Residential area (city/countryside)	43/8	12/4	0.232
Diabetes (no/yes)	23/38	7/9	0.878
Smoking (no/yes)	42/19	7/9	0.085
Cholelithiasis (no/yes)	53/8	16/0	0.193
Chronic liver disease (no/viral/others)	32/13/16	10/2/4	0.688
ICGR15 (%)	12±7	14±8	0.201
CEA (ng/ml)	42±176	5±4	0.444
CA19-9 (mAU/ml)	3583±12601	755±1880	0.127
Alpha fetoprotein level (ng/ml) (n=126)	76±352	45±139	0.640
Macroscopic finding of ICC * MF/MF+PDI/PDI/IP	20/33/5/3	8/5/2/1	0.527
Intrahepatic metastasis (no/yes)	45/16	11/5	0.362
Size of tumor (cm)	5.6±3.6	6.7±8.4	0.610
Vascular infiltration (no/yes)	54/7	11/5	0.120
Organ infiltration (no/yes)	44/17	13/3	0.534
Node metastasis (no/yes)	31/30	9/7	0.805
Severity of fibrosis (Staging) (n=197)	0.8±1.0	1.1±1.4	0.645
Japan TNM stage (I/II/III/IVA/IVB)	5/5/11/9/31	1/0/7/2/6	0.339
Extent of hepatectomy (partial resection/ segmentectomy/ hemihepatectomy)	9/48/4	5/11/0	0.211
Histological differentiation (well/moderately/poorly)	14/40/7	2/9/5	0.134
Postoperative complications			
hepatic failure (no/yes)	56/5	16/0	0.578
Postoperative ICC recurrence (no/yes)	15/46	6/10	0.308

Data are mean±SD or number of patients. #Multiple carcinoma (more than two cancers) were detected in only 2 of all 16 patients with multiple cancers. *Macroscopic findings according to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer in Japan. MF, Mass forming; PDI, periductal infiltration; IG, intraductal growth (13); CEA, carcinoembryonic antigen; ICGR15, indocyanine green retention rate at 15 min.

Table III. *Comparison between clinicopathological features and tumor recurrence in BDC patients with or without multiple cancers.*

	Solitary (n=74)	Multiple (n=10)	*p*-Value
Age (years)	67±13	70±10	0.548
Gender (males/females)	55/19	7/3	0.669
Atomic bomb survival (no/yes)	59/15	6/4	0.223
Occupation (none or office worker/manufacturing/ laborer)	53/8/13	6/2/2	0.692
Residential area (city/countryside)	61/13	8/2	1.0
Diabetes (no/yes)	48/26	9/1	0.156
Smoking (no/yes)	37/37	4/6	0.739
Cholelithiasis (no/yes)	64/10	8/2	0.630
Alcohol (no/chance drinker/heavy drinker)	36/21/17	4/3/3	0.848
Family history of biliary tract carcinoma (no/yes)	72/2	10/0	1.0
Chronic liver dysfunction (no/yes)	59/15	10/0	0.196
CEA (ng/ml)	4.4±5.3	5.0±5.5	0.560
CA19-9 (mAU/ml)	352±918	75±122	0.161
Macroscopic finding of ECC * (flat/nodular/ ulcer/papillary)	29/37/2/6	4/2/0/4	0.023
Size (cm)	3.0±1.1	3.6±1.2	0.239
Appearance of non-tumorous bile duct lumen (normal/ inflammatory)	40/34	7/3	0.719
Vascular infiltration (no/yes)	59/15	10/0	0.196
Node metastasis (no/yes)	32/42	8/2	0.042
Histological differentiation (papillary/well/ moderately/poorly)	4/28/24/18	3/3/3/1	0.061
Superficial epithelial infiltration of tumor (no/yes)	55/19	8/2	1.0
Organ invasion (no/yes)	43/31	9/1	0.081
Japan TNM stage (I/II/III/IVa/IVb)	4/11/18/41	1/6/0/3	0.005
Curability (R0/R1/R2)	18/27/29	5/4/1	0.119
Pancreatobiliary maljunction (no/yes)	71/3	9/1	0.404
Resection (hepatectomy/ pancreatectomy/ HPD/others)	21/42/5/6	3/4/2/1	0.502
Postoperative recurrence (no/yes)	42/32	9/1	0.080

Multiple carcinoma (more than two cancers) were detected in only 2 of all 10 patients with multiple cancers. *Macroscopic findings according to the Classification of Biliary Tract Carcinoma in Japan (14). Abbreviations as in tables I and II.

Table IV. *Comparison between clinicopathological features and tumor recurrence in GBC patients with or without multiple cancers.*

	Solitary (n=60)	Multiple (n=12)	*p*-Value
Age (years)	64±15	68±14	0.677
Gender (males/females)	28/32	4/8	0.531
Atomic bomb survival (no/yes)	58/2	11/1	1.0
Occupation (n=38) (no or office worker/ manufacturing/laborer)	19/1/8	8/0/2	0.699
Residential area (city/countryside)	42/18	6/6	0.101
Diabetes (no/yes)	51/9	8/4	0.210
Smoking (no/yes)	32/28	9/3	0.10
Alcohol (no/chance drinker/heavy drinker)	40/13/7	8/3/1	0.928
Chronic liver dysfunction (no/yes)	60/0	11/1	0.167
CEA (ng/ml)	5.9±7.6	3.8±4.7	0.665
Location of tumor in gall bladder (fundus/body/ neck/cystic duct)	29/15/14/2	8/3/1/0	0.545
Macroscopic finding of ICC * (flat/nodular/papillary/others)	7/16/21/16	2/2/6/2	0.659
Size (cm)	5.4±4.9	3.2±1.7	0.063
Vascular infiltration (no/yes)	51/9	12/0	0.339
Node metastasis (no/yes)	28/32	6/6	1.0
Japan TNM stage (I/II/III/IVa/IVb)	14/6/11/29	4/2/2/4	0.809
Resection (hepatectomy/ pancreatectomy/HPD/others)	17/3/2/38	2/0/0/10	0.361
Curability (R0/R1/R2)	39/16/5	9/1/2	0.319
Histological differentiation (papillary/well/moderately/ poorly/others)	20/15/9/9/7	3/2/3/3/1	0.770
Postoperative HCC recurrence (no/yes)	16/44	4/8	0.727

Multiple carcinoma (more than two cancers) were not detected in patients with multiple cancer. *Macroscopic findings by the Classification of Biliary Tract Carcinoma in Japan. (14). Abbreviations as in tables I and II.

Table V. *Comparison between clinicopathological features and tumor recurrence in PC patients with or without multiple cancers.*

	Solitary (n=92)	Multiple (n=5)	*p*-Value
Age (years)	65±12	67±3	0.965
Gender (males/females)	53/39	4/1	0.393
Atomic bomb survival (n=17) (no/yes)	12/0	4/1	0.755
Occupation (n=17) (no or office worker/ manufacturing/ laborer)	8/0/4	2/1/2	0.238
Residential area (n=17) (city/countryside)	7/5	3/2	0.793
Diabetes (n=31) (no/yes)	12/19	3/2	0.603
Smoking (n=31) (no/yes)	7/24	4/1	0.023
Alcohol (n=36) (no/chance drinker/heavy drinker)	11/14/11	4/1/0	0.086
CEA (ng/ml)	18±90	2.6±1.5	0.992
CA19-9 (mAU/ml)	620±2330	24±24	0.095
DUPAN-2 (U/ml)	456±558	139±184	0.193
Tumor location in the pancreas (head/body/tail)	57/26/9	1/3/1	0.176
Macroscopic finding of ICC *(mass forming/ invasive/cystic/others)	38/30/17/7	3/1/1/0	0.579
Size (cm)	3.8±1.5	1.8±0.4	0.0012
Tumor location (head/body/tail)	54/23/10	1/2/1	0.838
Vascular infiltration (no/yes)	63/29	4/1	1.0
Node metastasis (no/yes)	36/56	3/2	0.388
Japan TNM stage (I/II/III/IV)	7/7/16/62	2/0/1/2	0.110
Resection (PD/DP/CP/TP)	62/25/ 4/1	2/1/2/0	0.152
Histological differentiation (papillary/well/moderately/ poorly/mucinous/others)	4/13/62/8/2/3	2/0/1/1/1/0	0.12
Postoperative recurrence (no/yes)	39/53	5/0	0.017

Multiple carcinoma (more than two cancers) were detected in 3 patients with multiple cancer. *Macroscopic findings by the Classification of Pancreatic Carcinoma (15). PD, Pancreaticoduodenectomy; DP, distal pancreatectomy; CP, central pancreatectomy; TP, total pancreatectomy. Abbreviations as in tables I and II.

n=84, 14%), gallbladder carcinoma (GBC; n=72, 12%) and pancreatic carcinoma (PC; n=97, 16%). Surgery included hepatectomy (n=395), pancreatectomy (n=154, including hepato-pancreatectomy, n=9) and other types of resection (n=55) and achieved removal of macroscopically evident main tumor(s) without leaving any residual tumor. MC were detected in 23 patients with HCC (9%), including 7 patients (31%) with >3 cancers (MC >3, one patient had 5 tumors), 16 patients with ICC (21%, including 1 with MC >3), 10 with BDC (12%, 2 with MC >3, including one with 5 tumors), 12 with GBC (17%, none had MC >3) and 5 with PC (5%, 3 patients had MC >3).

The proportions of males afflicted with SC (68%) and MC (73%) were not significantly different (p=0.56). The mean age of patients of the MC group (69.6±7.9 years) was significantly higher than that of the SC group (65.2±12.3, p=0.038).

We examined the relationship between clinical history and MC. The study group included 86 patients (17%) who were atomic bomb survivors in Nagasaki, representing part of the 507 patients who could be examined for history of Nagasaki atomic bomb. The proportion of Nagasaki atomic

bomb survivors in the MC group (21 of 56 patients, 38%) was significantly higher than the SC group (79 of 451, 18%; $p<0.001$).

With respect to patient occupation, the study included unemployed or office workers (n=224), workers at manufacturing industry (n=26) and other workers (n=40) among 290 patients who could be examined for history of occupation. The proportions of patients working in the manufacturing industry (18 of 226 patients *vs*. 8 of 64) or other industries (31 of 226 *vs*. 9 of 64) were not significantly different between the SC and MC groups (8% *vs*. 13% and 14% *vs*. 14%, respectively; $p=0.382$ and 1.0). With respect to the residential area, 244 lived in the city and 314 lived in the countryside, while the area of residence could not be determined in 70. There was no significant difference in the residential area between the SC and MC groups (53 of 248 *vs*. 16 of 66 or 21 *vs*. 24%, $p=0.739$). With respect to diabetes mellitus (n=136 among 343 patients who could be examined), the proportion of diabetes mellitus was not significantly different between the SC (109 of 277 patients) and MC groups (27 of 66) (39% *vs*. 41%, $p=0.926$). With respect to smoking habit (n=150 among 343 patients who could be examined), the proportion of smokers was not significantly different between the SC (122 of 277 patients) and MC groups (28 of 66) (44 *vs*. 42%, $p=0.920$).

Twenty-three patients with MC all had HCC, in addition to gastric carcinoma in 6, head and neck carcinoma in 5, lung cancer in 5, colonic carcinoma in 3, esophageal carcinoma in 3, intrahepatic cholangiocarcinoma in 2, pancreas carcinoma in 2, urinary bladder carcinomas in 2, malignant lymphoma in one and carcinoma of kidney, uterus, thyroid, bile duct, gallbladder, submandibular gland, duodenum in one of each. Sixteen patients with MC had ICC, in addition to colon carcinoma in 5, lung cancer in 4, HCC in 2, gastric cancer in 2 and carcinoma of the esophagus, mammary gland, thyroid, thymus in one of each. Ten patients with MC had BDC, in addition to breast cancer in 2, gastric cancer in 2, lung cancer in 2, hepatoblastoma in one and carcinoma of gall bladder, uterus and urinary bladder in one of each. Ten patients with MC had GBC, in addition to gastric cancer in 4, breast cancer in 2 and cancer of colon, lung, uterus, kidney, HCC in one of each. Four patients with MC had PC, in addition to colon cancer in 3, gastric cancer in 3, thyroid cancer in 2, rectal carcinoid in one, pancreatic endocrine tumor in one and prostate carcinoma in one. Among the multiple carcinomas, the most frequent cancers were of the stomach in 17, colon in 12 and lung in 12.

Tables I-V show comparisons of the clinicopathological features and tumor recurrence rate after surgery between SC and MC groups for each cancer. Patients with HCC of the MC group were significantly older than those of the SC group ($p<0.01$). The proportion of atomic bomb survivors among the MC group was significantly higher than in the SC group ($p<0.01$). Comparison of double cancers and MC more than 3 cancers showed significantly higher proportion of atomic bomb victims among the MC more than 3 cancers than double cancers ($p<0.01$). Other background characteristics and liver function tests did not correlate with presence of MC. In HCC tumor-related factors, the proportion of patients with confluent or infiltrative nodular HCC was significantly lower in the MC group than the SC group ($p<0.05$). The HCC tumor was significantly smaller in the MC group than the SC group ($p<0.01$). Tumor stage was not different between the SC and MC groups. Tumor recurrence rate was not different between the groups.

Patients with ICC were significantly older in the MC group than the SC group ($p<0.05$). The proportion of atomic bomb survivors was significantly higher in the MC group than the SC group ($p<0.01$). The proportion of smokers tended to be significantly higher in the MC group than the SC group, albeit insignificantly. ICC tumor-related parameters and stage were not significantly different between the SC and MC groups. Tumor recurrence rate was not different between the two groups.

The demographics of patients with BDC were not significantly different between the SC and MC groups. The proportions of flat and nodular BDC tumors were significantly lower in the MC group than the SC group ($p<0.05$). The proportions of patients with node metastasis and advanced stage of BDC tumor were significantly lower in the MC group than the SC group ($p<0.05$). The proportions of patients with poorly-differentiated BDC or organ invasion tended to be lower in the MC group than the SC group, albeit insignificantly. Postoperative tumor recurrence tended to be lower in the MC group than the SC group but the difference was not significant.

GBC tumors tended to be smaller in the MC group than the SC group, albeit insignificantly. Other tumor-related factors and tumor recurrence rate were similar between the two groups.

Smokers formed a significantly smaller group in the MC group of patients with PC compared to the SC group ($p<0.05$). Heavy alcohol drinking was less common among patients of the MC group than the SC group, although statistically insignificant. Serum CA19-9 levels tended to be lower in the MC group than in the SC group but the difference was not significant. The tumor size was significantly smaller in the MC group than the SC group ($p<0.01$). Other PC tumor-related parameters were not significantly different between the two groups. No tumor recurrence was observed in the MC group compared with the SC group ($p<0.05$).

Figures 1-5 show comparison of disease-free and overall survival rates between SC and MC groups for each cancer type. In HCC patients, the disease-free (3-year survival rates for the SC and MC groups were 41 and 59%, while the mean survival times were 45 and 55 months, respectively) and overall survival rates (5-year survival rates for the SC

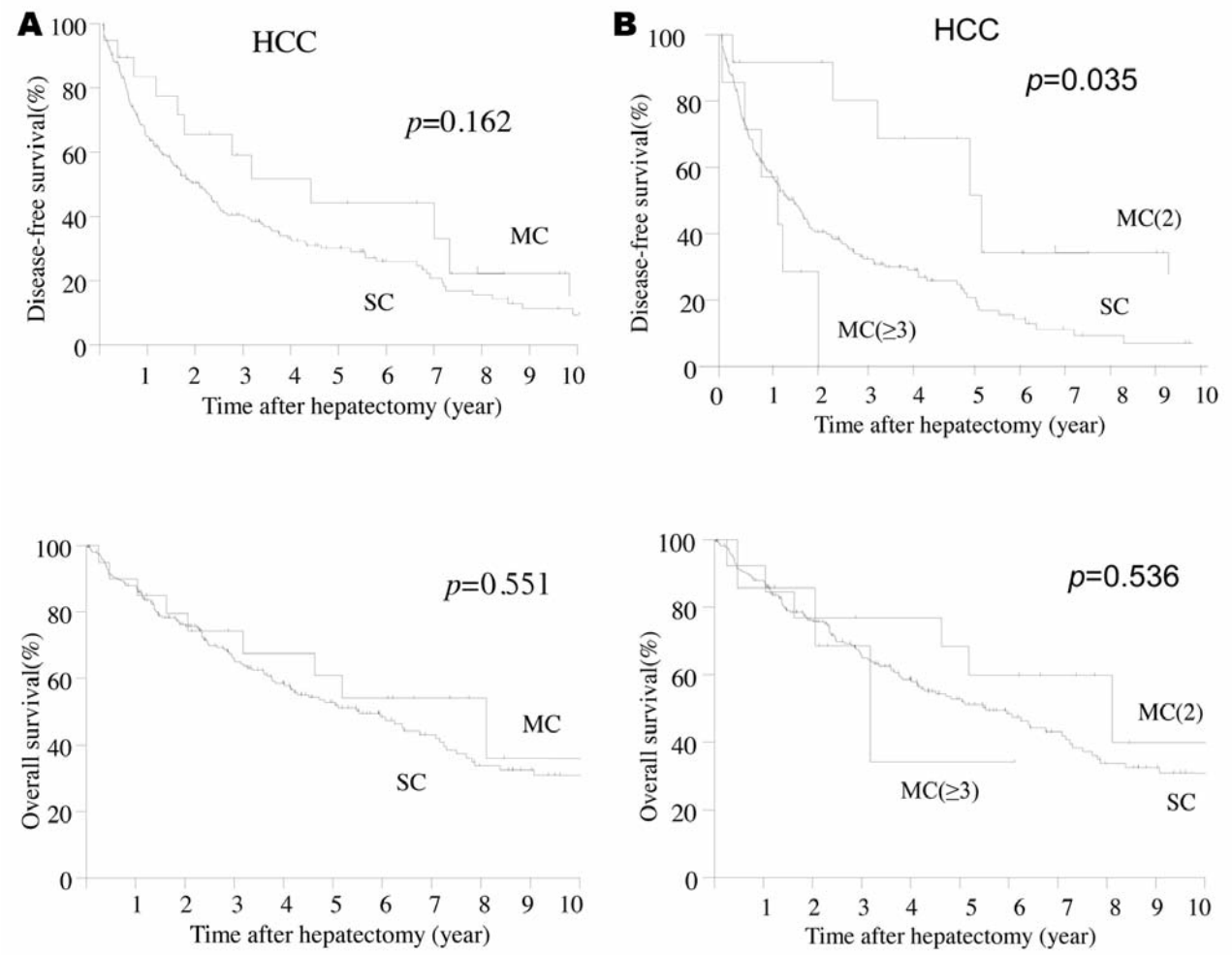

Figure 1. *(A) Disease-free and overall survival rates in patients with HCC for the single-cancer (SC) and multiple-cancer (MC) groups. (B) Disease-free and overall survival rates in patients with HCC for groups between the single-cancer (SC), double-cancer and multiple-cancer (MC) more than 3 cancers.*

and MC were 52 and 61%, while the mean survival times were 85 and 78 months, respectively) were not significantly different between the two groups (Figure 1A and B). In ICC patients, the disease-free (3-year survival rates for the SC and MC were 26 and 28%, while the mean survival times were 37 and 48 months, respectively) and overall survival rates (5-year survival rates for the SC and MC were 23 and 42%, while the mean survival times were 44 and 90 months, respectively) were not significantly different between both the two groups (Figure 2). For BDC, the disease-free (3-year survival rates for the SC and MC were 14 and 39%, while the mean survival times were 23 and 43 months, respectively) and overall survival rates (5-year survival rates for the SC and MC were 35 and 63%, while the mean survival times were 52 and 56 months, respectively) were not significantly different between the two groups (Figure 3). In GBC patients, the disease-free survival (3-year survival rates for the SC and MC were 55 and 66%, while the mean survival times were 69 and 111 months, respectively) tended to be better in the MC group than the SC group albeit statistically insignificant. The overall survival (5-year survival rates for the SC and MC were 38 and 100%, while the mean survival times were 49 and 62 months, respectively) was significantly better in the MC group than the SC group (Figure 4). In PC patients, the disease-free (3-year survival rates for the SC and MC were 18 and 100%, while the mean survival times were 45 and 194 months, respectively) and overall survival rates (5-year survival rates for the SC and MC were 25 and 100%, while the mean survival times were 31 and 193 months, respectively) were significantly better in the MC group than the SC group ($p<0.05$) (Figure 5).

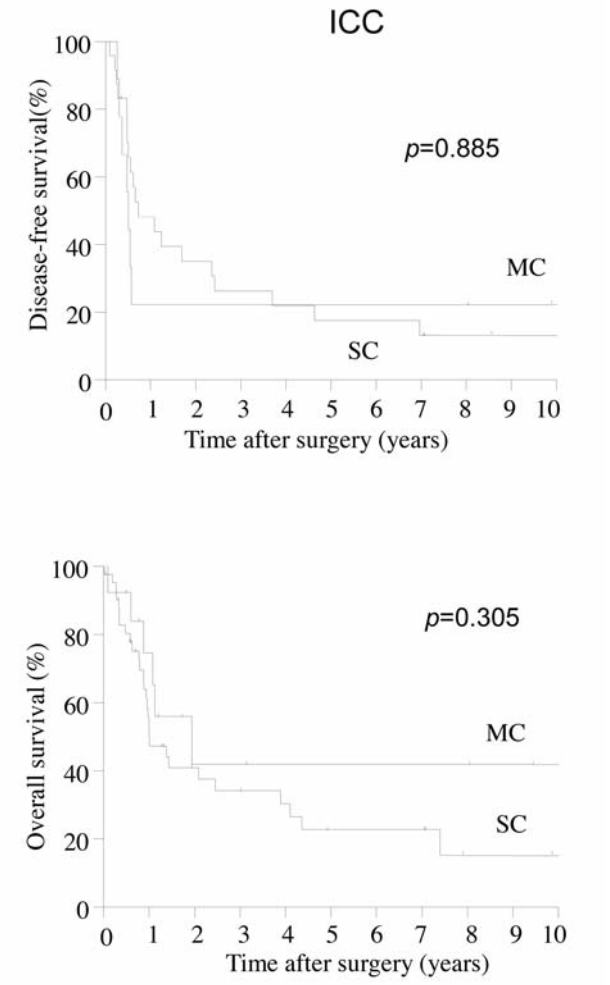

Figure 2. *Disease-free and overall survival rates in patients with ICC for the SC and MC group.*

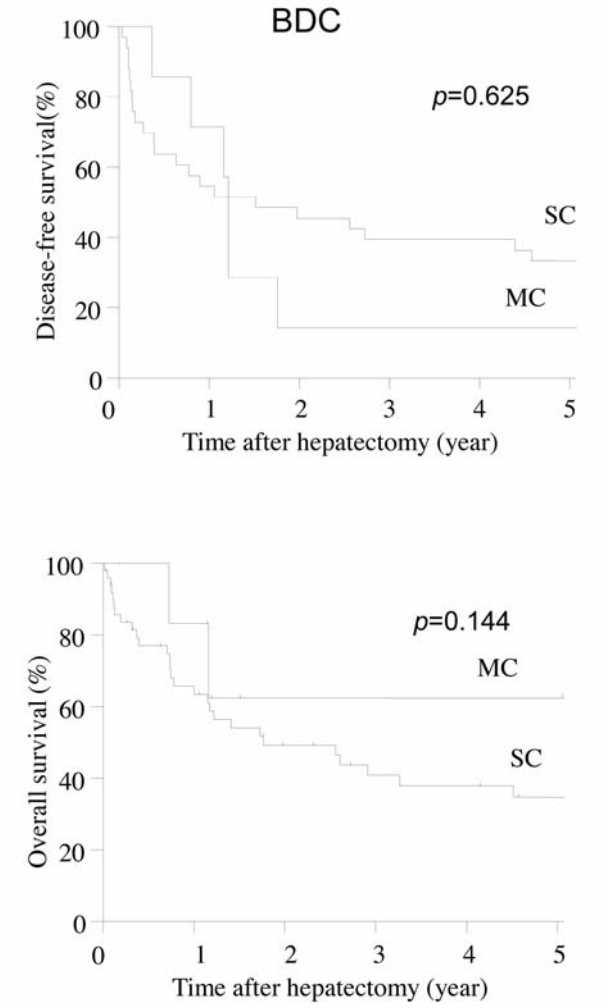

Figure 3. *Disease-free and overall survival rates in patients with BDC for the SC and MC groups.*

Discussion

In recent years, an increase in the number of patients with MC, following improvement in treatment of various solitary malignancies, has been witnessed (16, 17). A similar trend was noticed in patients with HBP malignancies, together with other concurrent malignancies (18-20). However, the actual status for digestive tract malignancies, especially with regard to multiple primary cancers, and the frequency of each cancer is not clear at present (21, 22). Particularly, in the field of hepato-biliary pancreas cancers, double and multiple primary cancers have been reported only in case reports (18-20). The present study was designed to clarify the clinicopathological characteristics and prognosis of multiple primary malignancies of hepato-biliary pancreas cancers. In the present series, the most frequent cancers encountered with HBP malignancies were gastric, colonic and lung, which are common cancers in Japan at present (Cancer Statistics in Japan; Table download by Center for Cancer Control and Information Services in National Cancer Institute in Japan; http:// ganjoho.jp/ pro/statistics/ en/ table_download.html).

In multiple primary cancers, hereditary factors, patient habits, metabolic co-morbidities and/or external exposure may influence multiple carcinogenesis (1-3, 8-12). With the exception of hereditary and gene-related carcinoma syndromes, the definite causes of sporadic multiple cancers remain poorly understood. Previous reports identified certain genetic or chromosomal aberrations in multiple primary cancers; however, the same findings could not be confirmed by others (23-25). In a previous genetic study, we reported that instability of chromosome 17 and p53 locus, detected by the fluorescence *in*

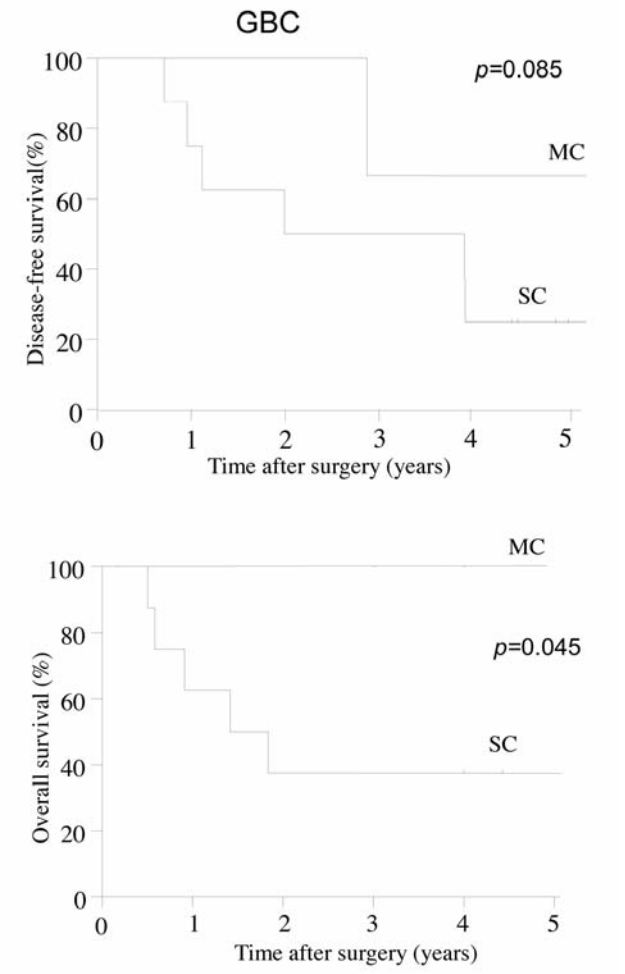

Figure 4. *Disease-free and overall survival rates in patients with GBC for the SC and MC groups.*

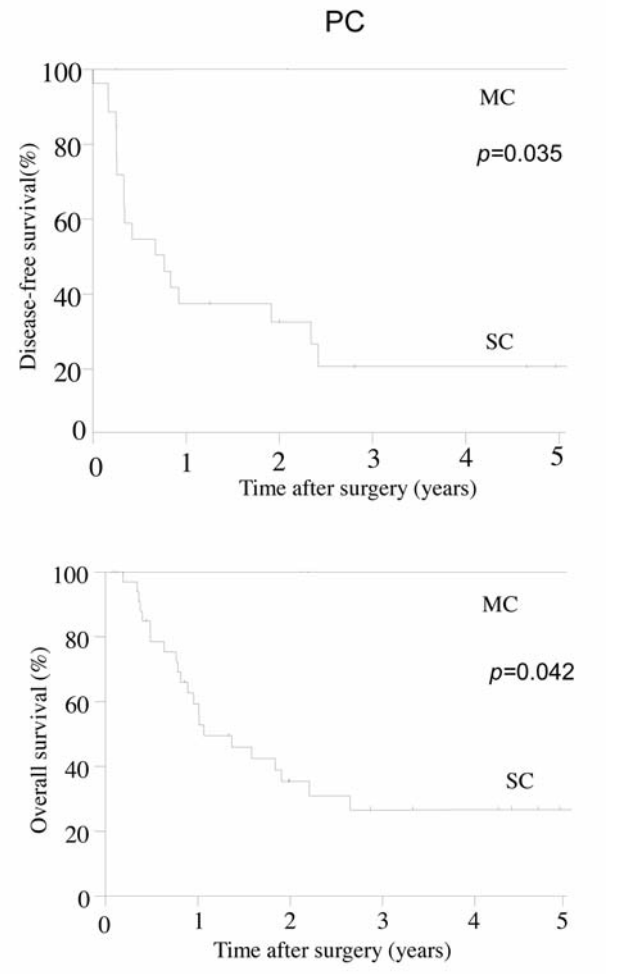

Figure 5. *Disease-free and overall survival rates in patients with PC for the SC and MC groups.*

situ hybridization (FISH) method, correlated with the presence of multiple primary colorectal carcinomas (26). Clinical application of special gene tests is still difficult and not popular in Japan so far, although detection of the *p53* mutation in blood is clinically simple (27). At this stage, clarification of the clinicopathological characteristics and patient background could help predict the occurrence of multiple primary carcinomas. Analysis of 597 patients showed that patients of the MC group were about 5 years older than those of the SC group. This tendency was observed in patients with HCC and ICC, while the age was similar to that of patients with primary liver cancer at diagnosis. Among 23 MC patients with HCC, 2 were treated before treatment of HCC, 8 were found to have HCC and MC at diagnosis and 13 were diagnosed after treatment of HCC. Thus, in many patients other cancers were observed at the time of diagnosing HCC or after. We speculate that certain genetic factors are triggered by aging to cause multiple carcinogenesis. Among 16 MC patients with ICC, 7 were treated before the diagnosis ICC, 2 were diagnosed simultaneously with MC and ICC and 7 were diagnosed with MC after treatment of ICC. In ICC, existing cancers might trigger the carcinogenic process of ICC or aging might trigger multiple carcinogenesis. How aging triggers cancer development is not clear at present, although sufficient evidence indicates that aging is related to carcinogenesis (28). Our hospital is located in Nagasaki city, where the proportion of patients who were exposed to radiation following the bombing of Nagasaki on August 1945 during the Second World War is higher compared to other cities in Japan. A significant proportion of patients with HCC and ICC who were atomic bomb survivors were diagnosed with MC. In our study, such proportion was significant for MC patients with three or more cancers. The incidence of leukemia and thyroid cancer increased significantly after the bombing (29, 30); however, the

relationship with other carcinomas or multiple cancers has not been examined systematically (31). Recent studies showed that DNA damage was still present in tissue specimens obtained from atomic bomb survivors (32, 33). Since 50-70 years have passed from the time of exposure to radiation, this survey considers this period as an appropriate time for multiple carcinogenesis, linking advanced age to MC. Although the relationship between radiation exposure and liver cancer is still unclear, it has been studied by few investigators (34, 35). Based on the metabolism in the liver and excretion in bile of various compounds (36), the relationship between exposure to organic solvents and carcinogenesis has been examined thoroughly and evidence indicates that exposure to certain solvents can cause cholangiocarcinoma in Japan. The relationship between occupation and residential area and carcinogenesis in hepatobiliary and pancreas cancers has been noticed; inhalation of high concentrations of such solvents at work or home can induce hepatotoxicity (37). Exposure of the liver to such carcinogens is relatively high (38). In the present study, there was no significant relationship between occupation and MC. However, among patients with MC, long exposure to various solvents by few who worked at or lived close to factories handling industrial organic solvents could be one cause of carcinogenesis. Personal habits were not significantly associated with MC in HBP malignancies. For example, while the smoking habit is a risk factor in several cancers including pancreatic cancer (39), it was not the same in patients with MC of ICC and PC in the present study. Multiple carcinogenesis in these carcinomas may be due to other factors.

The relationship between severity of each feature of malignancy, such as histopathology and occurrence of MC, was examined in each type of HBP malignancy in the present study. Kozawa *et al*. reported a better survival of patients with multiple sarcomas (40). There is no information at present on the prognosis of patients with MC in HBP malignancies; however, our results showed that the confluent nodular type was less common in HCC with MC, with this type being associated with poor prognosis of patients with HCC compared to the single nodular type (41). Furthermore, HCC in the MC group was smaller in size compared to the SC group. Thus, HCC in the MC group might represent a less severe malignancy compared with SC. However, this difference did not translate into a better survival suggesting that other tumor-related causes or liver function might influence patient survival. In ICC patients, clinicohistopathological factors did not correlate with prognosis. This finding could be related to the fact that the malignant behavior of ICC is high and prognosis is poor (excluding intraductal tumors (42)), which could minimize the importance of tumor features. In BDC patients, the MC group showed milder features of malignancy and a larger proportion of patients of the MC group had intraductal papillary tumors, which correlates with low malignancy (43). These findings tend to reflect a lower tumor recurrence rate rather than survival. In GBC patients, although there were no significant differences in tumor characteristics, apart from tumor size, the survival of patients of the MC group was significantly better than that of the SC group. In patients with PC, although there were no significant differences in malignancy characteristics, apart from tumor size and tumor marker level, the survival of patients of the MC group was significantly better than that of the SC group, similar to the GBC patients. Both GBC and PC were associated with high scores for malignant characteristics and poor survival (44, 45); however, survival in both types of malignancies in the MC groups was better than in the SN group. The reasons for the better survival cannot be explained by the parameters recorded in the present study. Furthermore, the tumor cellular activity in the MC group, such as genetic characteristics, is probably different. This gap in information calls for further studies to clarify the molecular characteristics of MC.

The present study has certain limitations. First, the subjects included in the study were only those who underwent surgical resection rather than all patients. Second, the study subjects were recruited from only a single cancer institution. A more comprehensive study of a larger number of subjects with more precise survey parameters is necessary. Third, the present study did not include any genetic or molecular studies; such studies are necessary to clarify the genetic features of MC. In modern era, complete cure of patients with HBP malignancy is potentially possible following the introduction of advanced treatment modalities. This, however, could potentially increase the proportion of patients with MC. Based on the results of the present work, prevention and prediction analysis of MC is required to be pursued in future studies. Unique to Japan, the harmful effects of irradiation in the aged survivals of Hiroshima and Nagasaki bombing should be studied to determine whether previous exposure is related to MC.

In conclusion, we reported the results of a survey on multiple carcinogenesis in patients with HBP malignancy who underwent surgical treatment. The results indicated that MC tended to occur in younger patients; however, when identified in elderly patients with HCC and ICC, it tended to be related to radiation exposure in the Second World War. Features of malignancy tended to be milder in BDC and PC of the MC group and the survival rates of patients with GBC and PC of the MC group were better than those of the single-cancer group. A better postoperative outcome after resection of such cancers would be expected. Careful follow-up for second or third occurrence of primary malignancies after primary curative treatment for HBP malignancy is necessary. Future studies of a larger number of patients are needed to determine the feasibility of genetic prediction to prolong survival of patients with MC.

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