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Clinical utility of Duke pancreatic monoclonal antigen type 2 in resectable biliary tract cancers

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ABSTRACT

This study aimed to investigate the clinical utility of Duke pancreatic monoclonal antigen type 2 (DUPAN-2) in addition to carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic Antigen (CEA) in biliary tract cancers (BTCs). Patients who underwent surgery for BTC were investigated. BTCs included five types: intrahepatic cholangiocarcinoma (ICC), hilar cholangiocarcinoma (HC), distal cholangiocarcinoma (DC), gall bladder carcinoma (GBC), and ampullary carcinoma (AC). The percentages of patients with elevated and normal tumor marker levels (high and normal groups) were determined across five BTC types, and recurrence-free survivals (RFSs) were compared between the two groups. Forty, 67, 56, 51, and 51 patients had ICC, HC, DC, GBC, and AC, respectively. In all patients, the percentages of patients with elevated tumor marker levels decreased in the order of CA19-9 (44.9%), DUPAN-2 (36.6%), and CEA (17.0%). This trend was consistent across four cancer types: ICC, HC, DC and GBC. In all patients, the high groups in three markers exhibited significantly poorer RFSs compared to the normal groups ($P < 0.01$, $P < 0.01$, $P < 0.01$, respectively). DUPAN-2 is a useful marker for recurrence in patients with resectable BTC.

Key words: *biliary tract cancer*, *duke pancreatic monoclonal antigen type 2*, *carbohydrate antigen 19-9*

INTRODUCTION

Tumor marker levels indicate the malignant potential of a tumor and survival period after surgery[1]. The most frequently used tumor marker in biliary tract cancer (BTC) is carbohydrate antigen 19-9 (CA19-9), followed by carcinoembryonic antigen (CEA). However, approximately 5–10% of patients have a Lewis antigen-negative phenotype and secrete very little or no CA19-9[2], and the phenotype is not generally measured due to the technical complexity and financial problem. Therefore, in patients with BTC with normal CA19-9 values, early-stage BTC or advanced BTC without elevation of CA19-9 levels can exist, making it challenging to estimate the malignant potential in patients with normal CA19-9 values. To overcome this problem, the efficacy of Duke pancreatic monoclonal antigen type 2 (DUPAN-2) has been reported in pancreatic cancer[3,4]. DUPAN-2 has an advantage, which can be used irrespective of the Lewis antigen phenotype. However, no studies have investigated DUPAN-2 in BTCs, and we investigated this marker in addition to CA19-9 and CEA.

METHODS

Study design

Patients' clinical data were collected through a retrospective review of prospectively maintained institutional databases.

Patient selection

Patients who underwent curative-intent surgery for BTC at the Department of Surgery, Hiroshima University Hospital, between April 2009 and April 2022 were investigated. BTCs include five cancers: intrahepatic cholangiocarcinoma (ICC), hilar cholangiocarcinoma (HC), distal cholangiocarcinoma (DC), gall bladder carcinoma (GBC), and ampullary carcinoma (AC).

Measurement of tumor markers

CA19-9, DUPAN-2, and CEA levels were simultaneously measured in patients diagnosed with BTC. Patients with jaundice underwent biliary drainage before surgery, and tumor markers were measured after their serum total bilirubin level had reduced to < 3.0 mg/dL.

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Exclusion criteria

Cases in which any of the three tumor markers were not measured were excluded. Additionally, conversion surgery cases for initially unresectable cancer with distant metastasis were excluded.

Classification of eligible patients

Normal serum CA19-9 level was defined as ≤ 37 U/mL, DUPAN-2 as ≤ 150 U/mL, and CEA as ≤ 5.0 ng/mL, based on the standard deviation in a normal population. Eligible patients were classified into two groups based on these upper normal limits: the normal and high groups for CA19-9, DUPAN-2, and CEA.

Pathological diagnosis

A pathological diagnosis of BTC was confirmed in all cases using surgically resected specimens.

Postoperative follow-up

Patients routinely underwent computed tomography scans every 3–4 months after curative-intent surgery. Recurrence was diagnosed when the recurrent tumor was radiographically evident.

Outcome measures

The following clinical parameters were investigated: i) clinicopathological features, including preoperative tumor marker levels, ii) percentage of patients with elevated tumor marker levels (high group), iii) recurrence-free survival (RFS) in the normal and high groups, and iv) overlap ratio of patients in the high groups.

Statistical analysis

Median values were calculated, and non-parametric statistical testing procedures were used. Categorical variables were compared using the chi-square or Fisher's exact test, as appropriate. Continuous variables were compared using the Mann–Whitney U test.

Multiple comparisons were performed among the five carcinomas. Additionally, we identified the pairs of groups that were significantly different when statistical significance was detected among the five cancer groups. RFS curves were established using the Kaplan–Meier method and compared using generalized Wilcoxon tests. All statistical analyses were performed using JMP statistical software version 13 (SAS Institute, Cary, NC, USA). Statistical significance was set at a P-value < 0.05.

RESULTS

Eligible patients

In total, 310 patients with BTC underwent curative-intent surgery between April 2009 and April 2022. Of these, 10 patients without CA19-9 measurements, 33 without DUPAN-2 measurements, and 2 with initially unresectable BTC with distant metastases were excluded. Among the remaining 265 patients, 40 (15.1%), 67 (25.3%), 56 (21.1%), 51 (19.2%), and 51 (19.2%) had ICC, HC, DC, GBC and AC, respectively.

Clinicopathological features

Table 1 presents the clinicopathological features of patients with BTCs. Regarding baseline characteristics, age and body mass index did not significantly differ among the five cancers. The GBC group had the largest proportion of female patients. Regarding preoperative tumor marker levels, the median levels of CA19-9 were 49.5, 43.2, 33.0, 37.0, and 11.0 U/mL in the ICC, HC, DC, GBC, and AC groups, respectively. The AC group had significantly lower CA19-9 levels than the ICC, HC, and DC groups. The median levels of DUPAN-2 were 130, 130, 57.2, 52.0, and 44.0 U/mL, respectively. The AC groups had significantly lower DUPAN-2 levels than the ICC and HC groups. The median levels of CEA were 3.1, 2.4, 2.8, 3.1, and 2.5 ng/mL, respectively; the levels showed no significant differences between the five cancer types. Regarding pathological findings, the proportion of advanced T3 or T4 cancer was highest in the GBC group, although the criteria of T factor were different in each cancers. The proportions of poorly differentiated carcinoma ranged from 2.5–7.8%; there were no significant differences between the five cancer types. The proportions of patients with lymph node metastases ranged from 32.5–46.4%; there were no significant differences.

Proportion of patients with elevated tumor marker levels (high group)

Fig. 1 depicts the proportions of patients with elevated tumor maker levels (high group). In all patients with BTC, the proportions of patients with elevated tumor marker levels decreased in the order of CA19-9 (44.9%), DUPAN-2 (36.6%), and CEA (17.0%). This trend was consistent in four cancer types, including ICC, HC, DC, and GBC, except for AC. In patients with these four cancers, the proportion of elevated CA19-9, DUPAN-2, and CEA levels ranged from 46.4% to 55.2%, 35.7% to 42.5%, and 10.7% to 25.0%, respectively. On the other hand, all three markers showed elevation in less than 30% of the patients in the AC group, and the percentage was highest in DUPAN-2 (27.5%).

RFS rate in all patients with BTCs

The median follow-up period for all patients was 29.7 months. The 1-, 2-, and 5-year RFS rates and median RFS were 87.1%, 77.2%, 68.5%, and not reached (NR) in the normal CA19-9 group and 72.6%, 53.4%, 40.8%, and 2.4 years in the high CA19-9 group, respectively (hazard ratio [HR]: 2.25, 95% confidential interval [CI]: 1.52–3.32, P < 0.001) (Fig. 2a). These values were 84.5%, 74.3%, 64.6%, and NR in the normal DUPAN-2 group and 73.8%, 53.5%, 41.8%, and 2.5 years in the high DUPAN-2 group, respectively (HR: 1.96, 95% CI: 1.33–2.88, P < 0.001) (Fig. 2b). These values were 84.2%, 71.9%, 60.0%, and 10.0 years in the normal CEA group and 62.8%, 40.4%, 36.3%, and 1.5 years in the high CEA group, respectively (HR: 2.28, 95% CI: 1.45–3.58, P < 0.001) (Fig. 2c).

Overlap ratio of patients in the high groups

Venn diagram shows the overlap of patients in the

Table 1 Clinicopathological features and tumor marker levels in five biliary tract cancers

Characteristics No. (%) or median (IQR)	**ICC** (*n* = 40)	**HC** (*n* = 67)	**DC** (*n* = 56)	**GBC** (*n* = 51)	**AC** (*n* = 51)	***P* value**
<Baseline characteristics>						
Age	72 (64.3–77.0)	73 (65.0–77.0)	72 (68.0–77.8)	74 (64–79)	71 (66–77)	0.91
BMI (kg/m^2)	22.8 (19.3–25.1)	22.1 (20.6–24.0)	22.6 (20.8–23.7)	22.8 (20.3–24.7)	22.2 (20.3–23.7)	0.99
Sex (male)	22 (55.0)	48 (71.6)[a]	39 (69.6)[b]	22 (43.1)[abc]	36 (70.6)[c]	0.007
<Preoperative tumor marker level>						
CA19-9 (U/mL)	49.5 (14.0–506.8)[d]	43.2 (10.0–254.0)[e]	33.0 (13.8–92.6)[f]	37.0 (6–186)	11.0 (4–33)[def]	0.005
DUPAN-2 (U/mL)	130 (46.5–520)[g]	130 (39–280)[h]	57.5 (26.3–260)	52.0 (24–300)	44.0 (24–210)[gh]	0.01
CEA (ng/mL)	3.1 (2.0–5.4)	2.4 (1.9–3.7)	2.8 (2.0–4.2)	3.1 (1.6–4.8)	2.5 (1.5–3.8)	0.37
<Pathological findings>						
Tumor differentiation (por)	1 (2.5)	11 (16.4)	8 (14.3)	4 (7.8)	4 (7.8)	0.19
UICC T stage (T3 and T4)	16 (40.0)	22 (32.8)	13 (23.2)	29 (56.8)	15 (29.4)	NA
LN metastasis	13 (32.5)	24 (35.8)	26 (46.4)	22 (43.1)	21 (41.2)	0.62
Number of metastatic LN	0 (0–1)	0 (0–1)	0 (0–2)	0 (0–2)	0 (0–2)	0.24

IQR, interquartile range; ICC, intrahepatic cholangiocarcinoma; HC, hilar cholangiocarcinoma, DC, distal cholangiocarcinoma; GBC, gall bladder carcinoma; AC, ampullary carcinoma; BMI, body mass index; CA19-9, carbohydrate antigen 19-9; DUPAN-2, Duke Pancreatic monoclonal antigen type 2; CEA, carcinoembryonic antigen; por, poorly differentiated carcinoma; LN, lymph node. [a–h], pairs with statistically significant differences ($P < 0.05$); NA, not applicable.

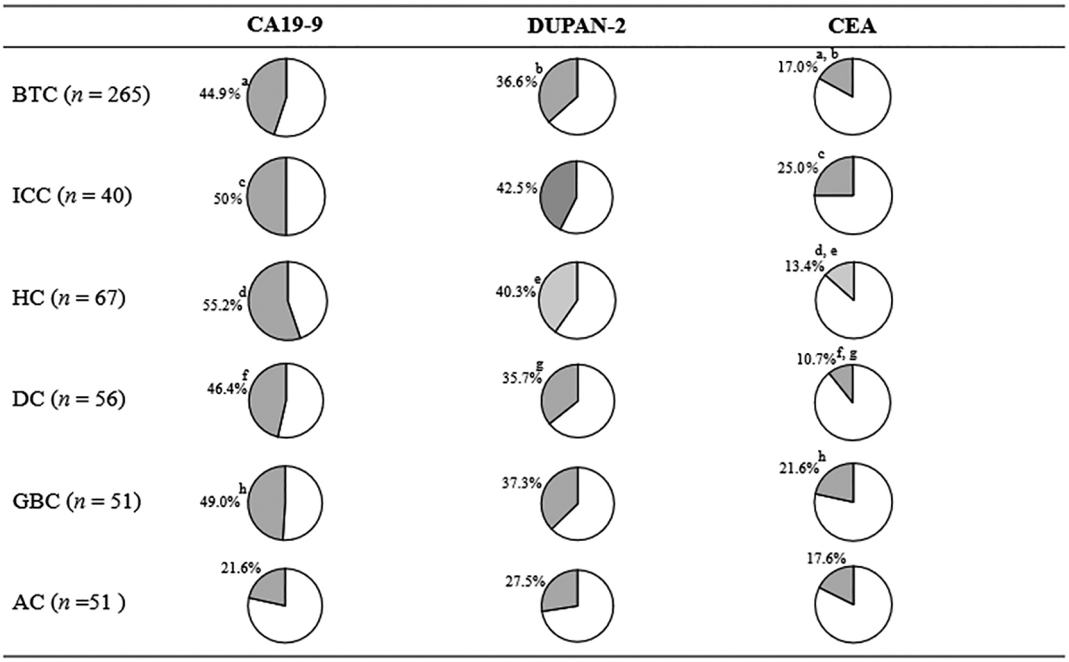

Figure 1 The proportions of patients with elevated tumor maker levels (high group). The gray areas of the pie graphs represent the proportions of patients with elevated tumor marker levels (high group). In contrast, the white areas represent those with normal tumor marker levels (normal group). [a–h], pairs with statistically significant differences ($P < 0.05$). CA19-9, carbohydrate antigen 19-9; DUPAN-2, Duke pancreatic monoclonal antigen type 2; CEA, carcinoembryonic antigen; BTC, biliary tract cancer; ICC, intrahepatic cholangiocarcinoma; HC, hilar cholangiocarcinoma, DC, distal cholangiocarcinoma; GBC, gall bladder carcinoma; AC, ampullary carcinoma.

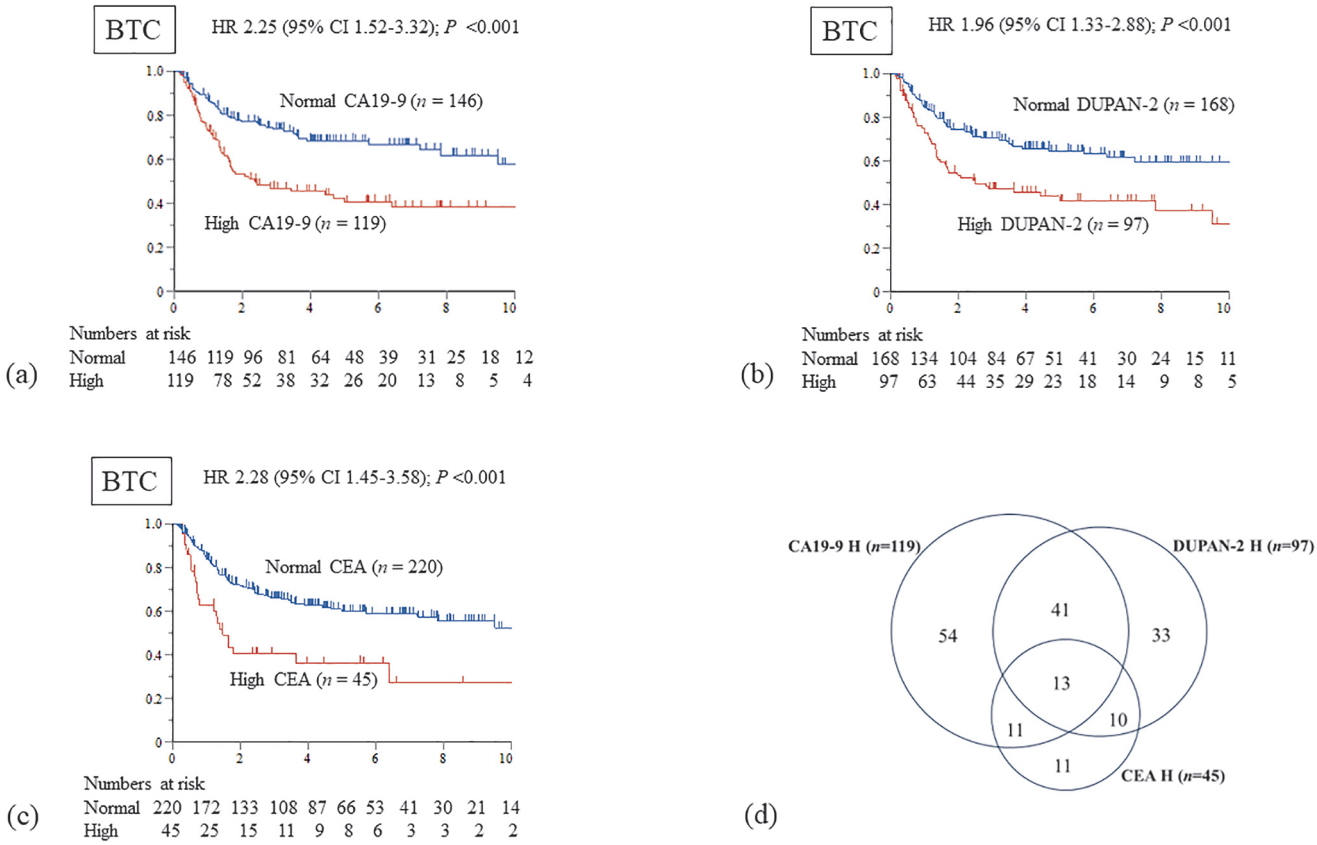

Figure 2 Recurrence-free survival curves in patients with biliary tract cancer and overlap ratio of patients in the high group. (a) Normal CA19-9 group *vs.* high CA19-9 group (b) Normal DUPAN-2 group *vs.* high DUPAN-2 group (c) Normal CEA group *vs.* high CEA group (d) Overlap ratio of patients in the high groups

high groups (Fig. 2d). Fifty-four (55.7%) among 97 high DUPAN-2 patients showed high CA19-9 levels, and they were correspond to 45.4% among 119 high CA19-9 patients. Twenty-four (53.3%) among 45 high CEA patients showed high CA19-9 levels, and they were correspond to 20.2% among 119 high CA19-9 patients.

DISCUSSION

BTCs have highly malignant potential, often leading to recurrences even after curative surgery. Therefore, tumor marker is expected to play a crucial role in identifying borderline resectable BTCs with little survival benefit of surgery. Our institution commenced routine measurements of DUPAN-2 levels, as well as CA19-9 and CEA levels in 2005; this study is the first to investigate DUPAN-2 in resectable BTCs. The following two important findings were identified in this study: i) different elevation ratios of tumor marker levels in each cancer, and ii) prognostic utilities of DUPAN-2 in BTCs.

The percentages of patients with elevated CA19-9, DUPAN-2, and CEA levels ranged from 44.9% to 55.2%, from 35.7% to 42.5%, and from 10.7% to 25.0% in four cancers except for AC (Fig. 1); the percentages decreased in the order of CA19-9, DUPAN, and CEA. However, the percentages weren't significantly different between CA19-9 and DUPAN-2. On the other hand, the patients with AC showed specific patterns. The elevated proportions of CA19-9 and DUPAN-2 levels were the lowest among the five cancers (21.6% and 27.5%, respectively); the proportion of DUPAN-2 was higher than that of CA19-9. The only one common finding in five canceres was that the percentage of elevated tumor marker level was lowest in CEA.

Regarding the prognostic utilities of three markers, the high group indicated significantly poor RFS in all three markers. Additionally, the RFS curves for CA19-9 and DUPAN-2 were similar. These results suggest that DUPAN-2 may be as useful as CA19-9 in predicting postoperative recurrence in resectable BTCs.

CA19-9 levels were within the normal range in more than half of the patients with BTCs. In patients with normal CA19-9 levels, Lewis-negative patients with advanced BTC can be included, making it challenging to estimate the malignant potential. Therefore, utilizing other markers, such as DUPAN-2 and CEA is important. In BTCs, the Ohio University group demonstrated the utility of the combination use of CA19-9 and CEA[6]. However, as shown in this study, the percentage of patients with elevated CEA is very low in BTCs, and only 20.2% of patients showed high CEA levels among the high CA19-9 patients. Therefore, CEA can not be used as the substitute for CA19-9, and DUPAN-2 is deemed to be superior to CEA in this role.

This study has some limitations. First, this study is based on data from a single-center database, and unexpected bias cannot be completely excluded. Second, the number of cases of each cancer type is small, necessitat-

ing a multicenter collaborative study.

In conclusion, DUPAN-2 is a useful marker for recurrence in patients with resectable BTCs.

Acknowledgements

All procedures performed were in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflicts of interest

The authors *declare that there are no* conflicts of interest.

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