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Tumor Marker Kinetics as Prognosticators in Patients with Unresectable Gallbladder Adenocarcinoma Undergoing Palliative Chemotherapy

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Background/Aims: To determine the prognostic value of carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9 in gallbladder cancer (GBC) during palliative chemotherapy. **Methods:** One hundred and twenty-three patients with pathologically confirmed unresectable GBC were included. Differences in serum CEA and CA 19-9 levels before and after chemotherapy were measured. Receiver operating characteristic curve analysis, Kaplan-Meier analyses of CEA, CA 19-9, and combined changes were performed to assess the optimal cutoff values and survival rates. **Results:** Patients with decreased tumor markers had significantly better progression-free survival (PFS) and overall survival (OS) than patients with increased tumor markers. The pre- and postchemotherapy CA 19-9 ratio had the highest area-under-the-curve values for predicting 3-month PFS and 1-year OS. In the multivariate analysis, increases in serum CA 19-9 during palliative chemotherapy in patients with unresectable GBC was an independent prognosticator of poor PFS and OS, with hazard ratios of 2.20 (p=0.001) and 1.67 (p=0.020), respectively. Patients with increases >10-fold were considered to have progressive disease, whereas individuals with increases >3-fold were likely to benefit from early imaging follow-up. **Conclusions:** CA 19-9 kinetics was a reliable prognosticator of PFS and OS in patients with unresectable GBC who underwent palliative chemotherapy. **(Gut Liver 2018;12:102-110)**

Key Words: Gallbladder neoplasms; CA-19-9 antigen; Carcinoembryonic antigen; Progression-free survival; Overall survival

INTRODUCTION

Gallbladder cancer (GBC) is the most common malignant tumor of the biliary tract. GBC is associated with a poor prognosis, with a 5-year overall survival (OS) rate of 18.5%, and only 20% of patients are eligible for resection at the time of diagnosis.[1] Palliative chemotherapy has shown survival benefits,[2] but the response rates are relatively low (17.1% to 36.6%).[3] The median survival time of patients with GBC is 4.6 to 11.7 months.[2,3]

In biliary tract cancer, serum carbohydrate antigen (CA) 19-9 and carcinoembryonic antigen (CEA) have been associated with prognosis.[4] Changes in these tumor markers during treatment were reported as prognosticators in pancreatic cancer[5-8] and cholangiocarcinoma.[9] For GBC, serum CEA and CA 19-9 are useful diagnostic[10-13] and prognostic[12,14-17] markers. However, previous studies employed spot measurements of CEA or CA 19-9, and studies evaluating the prognostic role of tumor marker kinetics in GBC have not been conducted.

The aim of the present study was to assess the prognostic values of serum CEA and CA 19-9 and their kinetics during palliative chemotherapy in patients with unresectable GBC.

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MATERIALS AND METHODS

1. Patients and study design

A single-center retrospective study was conducted in patients with unresectable GBC, which was pathologically confirmed as adenocarcinoma between January 2005 and April 2015 at Seoul National University Hospital. Patients who underwent at least four cycles of palliative chemotherapy and who had baseline and postchemotherapy tumor marker records were included. Patients with other malignant tumors diagnosed previously, a history of systemic chemotherapy, or normal pre- and post-chemotherapy tumor marker values were excluded (Fig. 1). The final analysis set included 123 patients.

All patients were followed up until 30 November 2015, and observations were censored at the time of death or loss to follow-up.

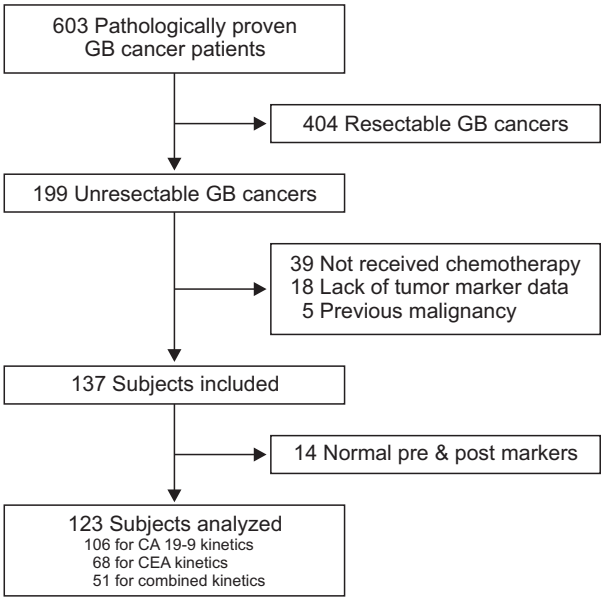

Fig. 1. Patient selection criteria.
GB, gallbladder; CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen.

Table 1. Baseline Characteristics of Eligible Patients

Characteristic	Value
Sex	
Male	62 (50.4)
Female	61 (49.6)
Age, yr	
≥65	57 (46.3)
<65	66 (53.7)
Drinking history	
Yes	31 (25.2)
No	92 (74.8)
Smoking history	
Yes	27 (22.0)
No	96 (78.0)
Symptoms	
Yes	113 (91.9)
No	10 (8.1)
CCI (cancer score subtracted)	
≥4.0	46 (37.4)
<4.0	77 (62.6)
Total bilirubin, mg/dL	
≥3.0	32 (26.0)
<3.0	91 (74.0)
CEA, ng/mL	
≥4.0	66 (46.3)
<4.0	57 (53.7)
CA 19-9, U/mL	
≥400	68 (55.3)
<400	55 (44.7)
Performance status (ECOG)	
0 or 1	103 (82.1)
2	22 (17.9)
Location	
Fundus and body	95 (77.2)
Neck	28 (22.8)
Metastasis	
Yes	78 (63.4)
No	45 (36.6)
Stage	
IIIb	30 (24.4)
IVa	15 (12.2)
IVb	78 (63.4)
Biliary drainage	
Yes	51 (41.5)
No	7 (58.5)
Chemotherapy	
Gemcitabine-based	99 (80.5)
Others	24 (19.5)
Concurrent radiotherapy	5 (4.1)
CEA_{change} (n=68)	1.41 (0.06–10.0)
$CA19\text{-}9_{change}$ (n=106)	1.00 (0.01–46.9)
$COMB_{change}$ (n=51)	1.19 (0.01–186)

Data are presented as number (%) or median (range).
CCI, Charlson comorbidity index; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9; ECOG, Eastern Cooperative Oncology Group; COMB, combination.

2. Data collection

Patient characteristics such as age, sex, symptoms at admission, and comorbid disease status (Charlson comorbidity index score)[18] were obtained. Variables in tumor characteristics including tumor location (fundus, body, and neck) and distant metastasis were collected.

Serum CEA and CA 19-9 levels at baseline and postchemotherapy were evaluated. Baseline levels were measured within 3 days before chemotherapy initiation (CEA_{pre} and CA $19\text{-}9_{pre}$). Postchemotherapy levels were measured within 3 days after the end of the second cycle of chemotherapy (CEA_{post} and CA $19\text{-}9_{post}$). Serum CEA and CA 19-9 were measured using a commercially available immunoradiometric assays (CA 19-9: IZO

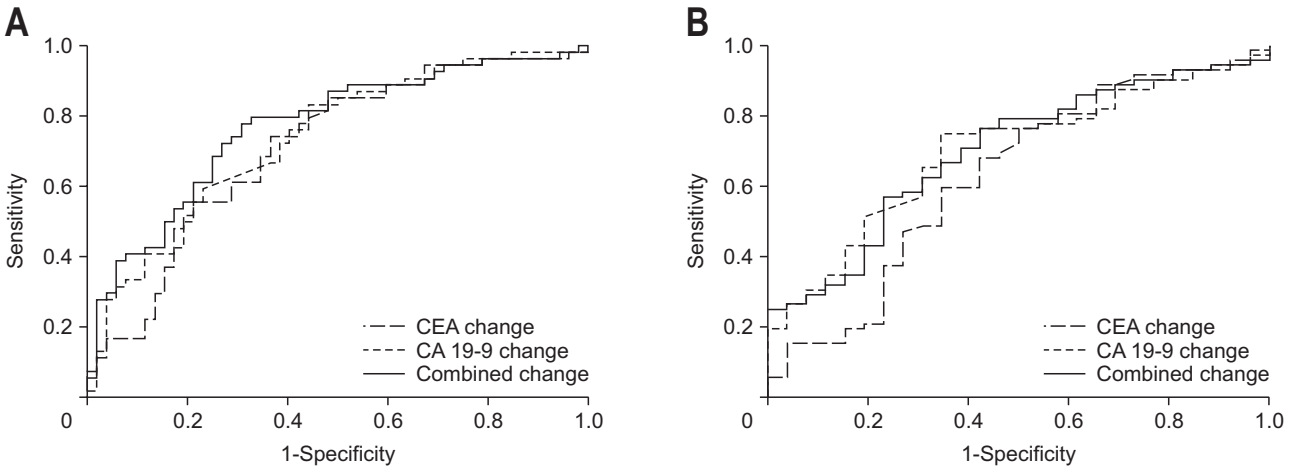

Fig. 2. Receiver operating characteristic (ROC) curve analysis of tumor marker kinetics as predictors of survival. (A) Progression-free survival. (B) Overall survival.
CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9.

Table 2. Sensitivity and Specificity for Predicting PFS and OS According to Serum Tumor Marker Changes after Chemotherapy

	PFS			OS		
	CEA_{change}	CA $19\text{-}9_{change}$	$COMB_{change}$	CEA_{change}	CA $19\text{-}9_{change}$	$COMB_{change}$
Cutoff 0.5						
Sensitivity, %	91.7*	84.2*	82.8*	82.8†	82.8†	82.8†
Specificity, %	31.2*	51.0*	50.0*	50.0†	50.0†	50.0†
<0.5	5.93±1.50‡	6.00±1.11‡	5.93±1.19‡	10.20±2.65‡	11.40±1.19‡	11.40±1.49‡
≥0.5	2.57±0.63‡	2.47±0.29‡	2.10±0.41‡	6.60±0.39‡	6.80±0.23‡	6.00±0.61‡
p-value	0.067	<0.001	0.009	0.258	0.001	0.011
Cutoff 1.0						
Sensitivity, %	83.3*	68.4*	72.4*	72.4†	72.4†	72.4†
Specificity, %	59.4*	69.4*	68.2*	68.2†	68.2†	68.2†
<1.0	5.87±0.36‡	5.60±0.79‡	5.30±0.78‡	11.40±0.80‡	9.60±1.12‡	8.93±2.16‡
≥1.0	2.31±0.36‡	2.17±0.27‡	2.10±0.23‡	6.17±0.50‡	6.63±0.43‡	6.17±0.52‡
p-value	0.002	<0.001	0.002	0.036	0.008	0.011
Cutoff 2.0						
Sensitivity, %	27.8*	45.6*	55.2*	55.2†	55.2†	55.2†
Specificity, %	85.4*	85.7*	72.7*	72.7†	72.7†	72.7†
<2.0	4.27±0.97‡	4.40±0.71‡	4.37±0.54‡	7.47±0.80‡	8.80±0.99‡	7.87±1.11‡
≥2.0	2.47±0.53‡	2.10±0.40‡	2.17±0.49‡	6.57±0.25‡	6.53±0.54‡	6.53±0.47‡
p-value	0.397	0.001	0.039	0.692	0.021	0.173

Data are presented as mean±SD.
PFS, progression-free survival; OS, overall survival; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9; COMB, combination.
*Sensitivity plus specificity for 3-month PFS; †Sensitivity plus specificity for 1-year OS; ‡Survival in months.

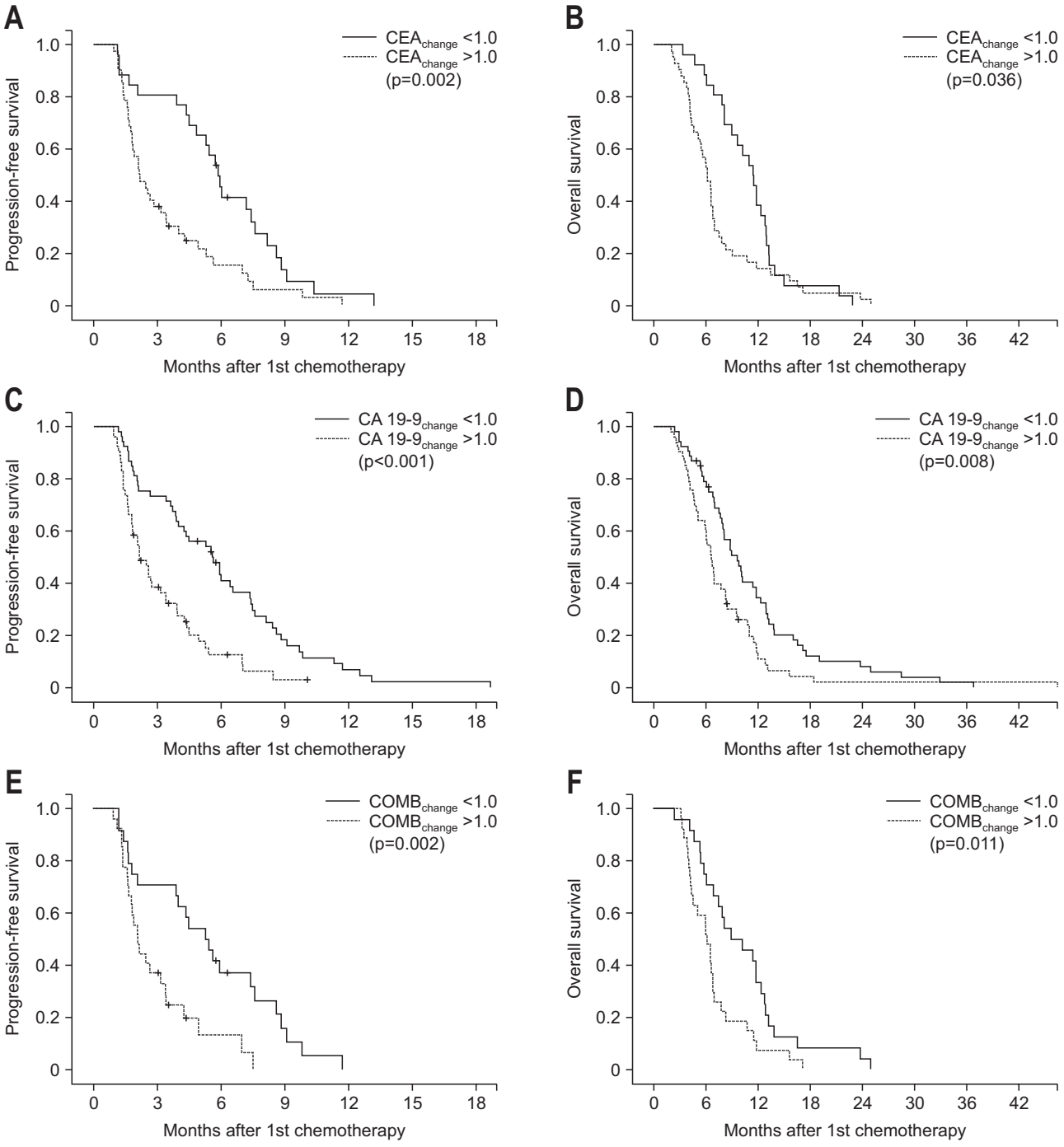

Fig. 3. (A-F) Kaplan-Meier plot of progression-free and overall survival according to serum tumor marker changes after chemotherapy using a cutoff value of 1.0.
CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9; COMB, combination.

TOP®, Institute of Isotopes Co., Ltd., Budapest, Hungary; CEA: RIAKEY®, Shinjin Medics Inc., Goyang, Korea). The initial total serum bilirubin levels and prothrombin times were also evaluated.

Data on progression-free survival (PFS) and OS were collected. Disease progression (PD) was assessed using abdominal computed tomography every two to four cycles of chemotherapy. PFS data was censored according to the date of loss to follow-up. The date of death was sourced from the records of the Korean Central Cancer Registry.

3. Statistical analyses

Tumor marker kinetics were defined as $CEA_{change}=\frac{CEApost}{CEApre}$ and $CA\ 19\text{-}9_{change}=\frac{CA\ 19\text{-}9post}{CA\ 19\text{-}9pre}$. Combined tumor marker kinetics were defined as $COMB_{change}=CEA_{change}\times CA\ 19\text{-}9_{change}$. The median, and

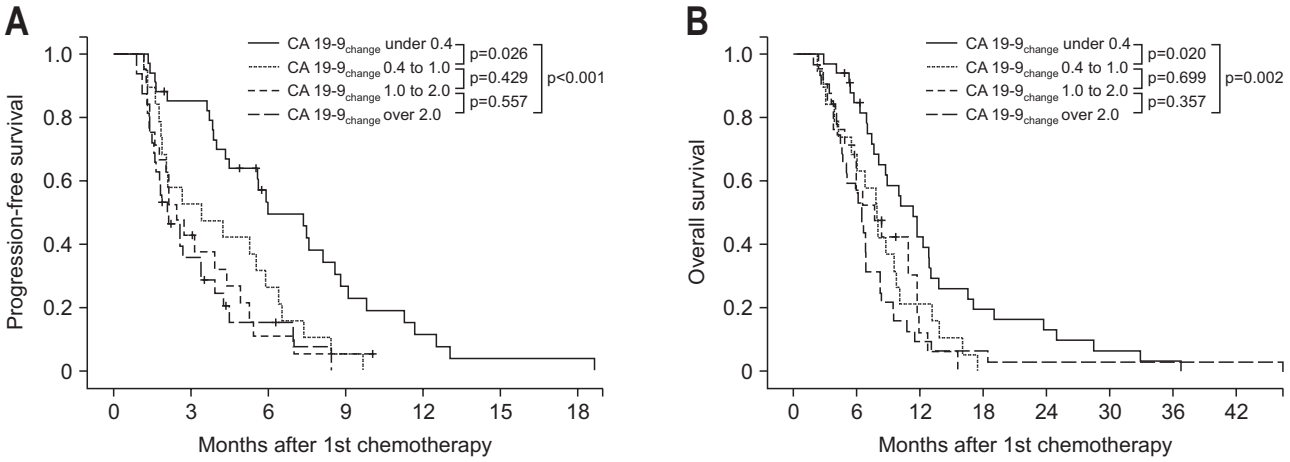

Fig. 4. Progression-free (A) and overall survival (B) according to changes in carbohydrate antigen (CA) 19-9.

Table 3. Correlations between Changes in CA 19-9 and Other Variables

	CA 19-9		p-value
	Decrease	Increase	
Sex			
Male	26	30	
Female	27	23	0.560
Age, yr			
≥65	27	23	
<65	26	30	0.560
Drinking			
Yes	16	12	
No	37	41	0.509
Smoking			
Yes	11	13	
No	42	40	0.817
CCI (cancer subtracted score)			
≥4.0	21	20	
<4.0	32	33	1.000
Symptoms			
Yes	47	50	
No	6	3	0.488
Location			
Fundus and body	35	45	
Neck	18	8	0.041
Distant metastasis			
Yes	31	39	
No	22	14	0.151
Biliary drainage			
Yes	25	19	
No	28	34	0.324
CEA, ng/mL			
≥4.0	25	27	
<4.0	28	26	0.846
CA 19-9, U/mL			
≥400	37	27	
<400	16	26	0.073

CA 19-9, carbohydrate antigen 19-9; CCI, Charlson comorbidity index; CEA, carcinoembryonic antigen.

first and third quadrant values, of the kinetic parameters were calculated.

Receiver operating characteristic (ROC) curve analysis of CEA_{change}, CA $19\text{-}9_{change}$, and $COMB_{change}$ was performed to assess 3-month PFS and 1-year OS rates. The cutoff value for the highest sum of sensitivity and specificity was used for further analyses. Kaplan-Meier analyses were performed for survival evaluation. The log-rank test was used to assess the relationships between tumor marker kinetic parameters and PFS or OS.

Univariate analysis with the log-rank test was conducted to compare survival using the cutoff values of the tumor marker kinetic parameters. Factors associated with survival in the univariate analysis with a p-value <0.10 were used in multivariate analysis. Cox regression analysis was performed to identify independent prognosticators.

Hazard ratios and 95% confidence intervals were calculated for each predictive factor. Two-sided p-values of <0.05 were considered statistically significant. All statistical analyses were performed with IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

1. Patient demographics

Baseline patient characteristics are listed in Table 1. The median age was 64 years (range, 25 to 85 years). Thirty patients had stage IIIb disease, all of which had unresectable disease due to extensive liver invasion or regional lymph node metastasis. The majority of patients underwent gemcitabine plus cisplatin chemotherapy (61.0%) with a mean duration of 4.8 (±3.0) cycles. Other chemotherapy regimens included TS-1 plus cisplatin (15.5%) with a mean duration of 5.2 (±3.1) cycles, gemcitabine plus oxaliplatin (13.8%) with a mean duration of 5.9 (±3.7) cycles, gemcitabine plus TS-1 (4.1%), infusional 5-fluorouracil, doxorubicin, and mitomycin-C (1.6%), capecitabine alone (1.6%), and TS-1 alone (2.4%). The median Charlson comorbidity index was 8 (range, 3–13). The median serum CEA and CA 19-9 levels were 3.9 mg/L (range, 0.5 to 1,350 mg/L) and 413 U/mL (range, 1 to 145,000 U/mL), respectively. The median interval between diagnosis and chemotherapy was 16 days (range, 0 to 123 days). The median PFS and OS were 3.9 and 8.1 months, respectively.

Table 4. Prognosticators of PFS in Patients with Unresectable Gallbladder Cancer

	Univariate			Multivariate		
	No.	PFS	p-value	HR	95% CI	p-value
Sex						
Male	62	2.7				
Female	61	4.8	0.095	0.81	0.53–1.23	0.317
Age, yr						
≥65	57	3.9				
<65	66	3.9	0.918			
Drinking						
Yes	31	4.0				
No	92	3.7	0.315			
Smoking						
Yes	27	3.9				
No	96	4.0	0.839			
Symptom						
Yes	113	3.9				
No	10	2.6	0.826			
Performance status (ECOG)						
0 or 1	101	4.0				
2	22	3.2	0.505			
Location						
Fundus and body	95	3.4				
Neck	28	4.5	0.076	0.79	0.46–1.38	0.410
Distant metastasis						
Yes	78	2.7				
No	45	5.7	0.018	1.45	0.92–2.29	0.115
Biliary drainage						
Yes	51	3.4				
No	72	4.8	0.171			
Total bilirubin, mg/dL						
≥3.0	32	3.4				
<3.0	91	4.3	0.336			
CEA, ng/mL						
≥4.0	66	3.2				
<4.0	57	4.4	0.066	1.44	0.91–2.29	0.112
CA 19-9, U/mL						
≥400	68	3.9				
<400	55	3.9	0.328			
Chemotherapy						
GP	75	4.0				
Others	48	3.4	0.851			
Interval between diagnosis and treatment, day						
≤15	59	4.0				
>15	64	3.9	0.433			
CA 19-9$_{change}$						
≥1.0	53	5.6				
<1.0	53	2.2	<0.001	2.20	1.39–3.47	0.001

PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9; GP, gemcitabine plus cisplatin.

2. Prognostic value of CEA$_{change}$, CA 19-9$_{change}$, and COMB$_{change}$

In the ROC analysis, the areas-under-the-curve of the CEA$_{change}$, CA 19-9$_{change}$, and COMB$_{change}$ for predicting 3-month PFS were 0.727, 0.750, and 0.734, whereas those for 1-year OS were 0.623, 0.742, and 0.720, respectively (Fig. 2).

The relationships between tumor marker kinetics and survival are shown in Table 2. CA 19-9$_{change}$ was significantly correlated with PFS and OS. However, CEA$_{change}$ was significantly correlated with PFS and OS only at the cutoff value of 1.0. The relationship between COMB$_{change}$ and PFS and OS was variable but significant at most values with the exception of a cutoff value of 2.0 for OS.

Survival curves according to tumor kinetic parameters using a cutoff value of 1.0 are shown in Fig. 3. The median PFS was 5.9 and 2.3 months in patients with a CEA$_{change}$ of <1 and ≥1, respectively (p=0.002). The median OS was 11.4 and 6.2 months in those with a CEA$_{change}$ of <1 and ≥1, respectively (p=0.036). The median PFS was 5.6 and 2.2 months in patients with a CA 19-9$_{change}$ of <1 and ≥1, respectively (p<0.001). The median OS was 9.6 and 6.6 months in patients with a CA 19-9$_{change}$ of <1

and ≥1, respectively (p=0.008). The median PFS was 5.3 and 2.1 months in those with a $COMB_{change}$ of <1 and ≥1 (p=0.002). The median OS was 8.9 and 6.2 months in those with a $COMB_{change}$ of <1 and ≥1, respectively (p=0.011).

CA 19-9_{change} was the most valuable prognostic marker. Kaplan-Meier analyses according to CA 19-9_{change} cutoff value of 0.4, 1.0, and 2.0 (which represent the first quadrant, median, and third quadrant) are shown in Fig. 4. Patients with a CA 19-9_{change} <0.4 had significantly better survival compared to those with greater changes. The linearity of the PFS and OS in were statistically significant (both p<0.001). Because CA 19-9_{change} was the most valuable prognosticator, we used CA 19-9_{change} as a marker in further analyses.

3. CA 19-9_{change} as a predictor of response to chemotherapy

Responses after four-cycle (rather than 3-month due to the variety of chemotherapy regimen) of chemotherapy were assessed according to the modified Response Evaluation Criteria in Solid Tumors (version 1.1). Sixteen patients (13.0%) had a partial response, 41 (33.3%) had stable disease, and 66 (53.7%) showed PD. A high CA 19-9_{change} was correlated with PD (p=0.001), but a low CA 19-9_{change} was not associated with partial response (p=0.500). Three patients with a CA 19-9_{change} >10.0 showed PD after four-cycle of chemotherapy, and 20 patients with CA 19-9_{change} >3.0, 17 (85%) of whom underwent four-cycle of chemotherapy showed PD.

4. Prognostic value of CA 19-9_{change} associated with PFS

The correlations of CA 19-9_{change} with other baseline variables were analyzed, but there were no significant correlations (Table 3). Univariate analysis revealed that male sex (p=0.095), a primary mass located in the gallbladder neck (p=0.076), positive distant metastasis (p=0.018), CEA ≥4.0 ng/mL at diagnosis (p=0.045), and CA 19-9_{change} ≥1.0 (p<0.001) had a p-value of <0.10 for PFS. However, CA 19-9_{change} ≥1.0 alone was an independent prognosticator of PFS (p=0.001) (Table 4).

5. Prognostic value of CA 19-9_{change} associated with OS

Univariate analyses revealed that male sex (p=0.058), an Eastern Cooperative Oncology Group performance score of 2 (p=0.050), distant metastasis (p=0.072), CEA ≥4.0 ng/mL (p=0.001), CA 19-9 ≥400 U/mL (p=0.007), and CA 19-9_{change} ≥1.0 (p=0.008) had a p-value of <0.10. Multivariate Cox regression analysis showed that baseline CEA ≥4.0 ng/mL, baseline CA 19-9 ≥400 U/mL, and CA 19-9_{change} ≥1.0 were independent prognosticators of OS (p=0.018, p=0.022, and p=0.020, respectively) (Table 5).

6. Effect of total serum bilirubin on CA 19-9 levels

The correlation between serum CA 19-9 and serum total bilirubin level, and that between CA 19-9_{change} and serum total bilirubin were not significant (p=0.155 and 0.845, respectively). We

Table 5. Prognosticators of OS in Patients with Unresectable Gallbladder Cancer

	Univariate			Multivariate		
	No.	OS	p-value	HR	95% CI	p-value
Sex						
Male	62	7.0				
Female	61	8.8	0.058	0.81	0.53–1.23	0.320
Age, yr						
≥65	57	6.9				
<65	66	9.5	0.130			
Drinking						
Yes	31	7.5				
No	92	8.1	0.473			
Smoking						
Yes	27	8.3				
No	96	7.8	0.419			
Symptoms						
Yes	113	7.9				
No	10	8.9	0.274			
Performance status (ECOG)						
0 or 1	101	8.3				
2	22	6.6	0.050	1.15	0.69–1.93	0.592
Location						
Fundus and body	95	7.8				
Neck	28	9.5	0.104			
Distant metastasis						
Yes	78	6.8				
No	45	9.6	0.072	1.35	0.87–2.11	0.186
Biliary drainage						
Yes	51	7.0				
No	72	8.3	0.669			
Total bilirubin, mg/dL						
≥3.0	32	7.0				
<3.0	91	8.1	0.763			
CEA, ng/mL						
≥4.0	66	6.8				
<4.0	57	9.6	0.001	1.76	1.10–2.80	0.018
CA 19-9, U/mL						
≥400	68	7.0				
<400	55	9.6	0.007	1.74	1.08–2.80	0.023
Chemotherapy						
GP	75	7.8				
Others	48	8.3	0.250			
CA 19-9_{change}						
≥1.0	53	6.6				
<1.0	53	9.6	0.008	1.67	1.08–2.58	0.020

OS, overall survival; HR, hazard ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9; GP, gemcitabine plus cisplatin.

Table 6. Survival Analysis of High- and Low-Serum Bilirubin Subgroups

	Bilirubin ≥3 mg/dL			Bilirubin <3 mg/dL		
	CA 19-9$_{change}$ <1 (n=18)	CA 19-9$_{change}$ ≥1 (n=11)	p-value	CA 19-9$_{change}$ <1 (n=35)	CA 19-9$_{change}$ ≥1 (n=42)	p-value
PFS, median, mo	4	2.7	0.296	5.9	2.2	<0.001
OS, median, mo	7.1	6.7	0.955	10.2	6.6	<0.001

CA 19-9, carbohydrate antigen 19-9; PFS, progression-free survival; OS, overall survival.

did a subgroup analysis which divided the subjects according to serum total bilirubin levels, and 29 out of 106 patients had a serum total bilirubin level >3.0 mg/dL (Table 6). The median PFS was 4.0 and 2.7 months in patients with a CA 19-9$_{change}$ of <1 and ≥1, respectively (p=0.296). The median OS was 7.1 and 6.7 months in patients with a CA 19-9$_{change}$ of <1 and ≥1, respectively (p=0.955).

DISCUSSION

In this retrospective study, we aimed to assess the prognostic value of serum tumor marker kinetics after chemotherapy in patients with unresectable GBC. The results revealed that tumor marker changes after first two cycles of chemotherapy were independent prognosticators of survival. Serum CEA, serum CA 19-9, or a combination of the two were valuable prognosticators; however, among them, CA 19-9 kinetics was the most valuable prognosticator of survival. In addition, the serum CA 19-9 level after two cycles of chemotherapy was a valuable predictor of PD after four cycles of chemotherapy. Therefore, we suggest that patients with >10-fold increase in serum CA 19-9 after two cycles of chemotherapy should be considered as having PD, and patients with >3-fold increase in serum CA 19-9 should be considered for early imaging studies.

Multivariate analyses in previous studies of patients with GBC that utilized specific cutoff values of spot serum CA 19-9 did not identify serum CA 19-9 as an independent prognosticator.[19-21] This could be explained by the wide variation in CA 19-9 secretion levels between GBC cases. Although the serum CA 19-9 levels vary between patients and may not represent tumor burden, the relative value of CA 19-9 in a single patient may be reflective of the tumor burden, regardless of the absolute CA 19-9 level. The present study showed that the relative change in CA 19-9 did not correlated with spot serum CA 19-9 measurements, and that the relative change was an independent prognosticator, similar to previous findings in pancreatic cancer[5-8] and cholangiocarcinoma.[9]

Yu *et al*.[14] focused on the change of serum CA 19-9 and CEA after resection of GBC. They showed that increased CA 19-9 and CEA after GBC resection were independent prognosticators of poor survival. Combined with postoperative pathology, which is the most important prognosticator, they classified postsurgical patients into different prognostic groups, which aided further decision making. Unlike patients with resectable GBC, patients with unresectable GBC undergo palliative chemotherapy and exhibit variable tumor responses, a valid assessment of such responses is critical in further decision making. The present study focused on the relationship between chemotherapy response and tumor marker changes, and identified criteria for early imaging follow-up or chemotherapy discontinuation based on these changes.

We excluded subjects with normal pre- and postchemotherapy tumor markers in this study and this may create some degree of selection bias. However, excluding such subjects does not facilitate any favorable tendency in the conclusion of this study. On the other hand, we could not conclude some issues regarding chemotherapy response due to the insufficient number of subjects. Despite tumor marker kinetics significantly correlated with progressive disease, we could not draw a conclusion correlating tumor marker kinetics with partial or complete response. Only 16 patients showed partial response to chemotherapy and there were no patients with complete response. Studies with a larger number of subjects are warranted to reach a conclusion.

High baseline serum total bilirubin is correlated with high baseline serum CA 19-9 level, and could therefore influence CA 19-9$_{change}$.[22] There is no universal agreement on how to adjust the serum CA 19-9 level. Kim *et al*.[23] suggested a formula correcting the effect of serum total bilirubin on serum CA 19-9 level, which divides serum CA 19-9 level with serum total bilirubin level. However, the serum total bilirubin threshold 2.0 mg/dL makes this formula inadequate to this study due to the measurable nature of tumor marker changes. Instead, we did a subgroup analysis, and 29 of 106 patients had a serum total bilirubin level >3.0 mg/dL (Table 6). High baseline serum bilirubin (>3.0 mg/dL) tended to be associated with improved survival in patients with a lower CA 19-9$_{change}$, although the association did not reach significance, most likely because of the small number of patients. Therefore, the influence of combined total bilirubin and serum CA 19-9 changes should be evaluated in further studies with a larger number of patients.

In conclusion, CA 19-9 kinetics is a valuable prognosticator of patient survival and treatment response during chemotherapy for unresectable GBC.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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