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ORIGINAL ARTICLE

Prognostic value of hypocholesterolemia in patients with gastric cancer

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ABSTRACT

Background: According to previous studies, low serum total cholesterol (TC) is associated with higher cancer incidence and mortality. However, the prognostic implications of preoperative TC in patients with gastric cancer (GC) remain to be determined.

Methods: A total of 1251 patients with GC, who underwent radical gastrectomy between 2005 and 2008, were recruited. Propensity score weighting (PSW) based on a generalized boosted method (GBM) was used to control for selection bias.

Results: After balancing the preoperative and operative covariates, low TC was associated with high incidence of complications (severe complication rate: 15.2% (Low TC) vs. 4.7% (Normal TC) vs 5.5% (High TC); p = 0.004). In multivariable analysis, lowering TC was associated with poor OS and RFS in weighted population. [OS: hazard ratio (HR) = 0.92; 95% CI = 0.867–0.980; P = 0.009 and RFS: HR = 0.93; 95% CI = 0.873–0.988; P = 0.02].

Conclusions: Preoperative TC is a useful predictor of postoperative survival and postoperative complications in patients with stage I–III GC and may help to identify high-risk patients for rational therapy, including nutritional support, and timely follow-up.

1. Introduction

Gastric cancer (GC) is one of the most common cancers of the gastrointestinal tract. It is the third most frequently diagnosed cancer after liver and lung cancer, and is the third leading causes of cancer mortality in worldwide.[1]

Early GC, which is more frequent in East Asia, is currently treated by minimally invasive surgery or endoscopic resection and shows favorable outcomes, while advanced GC, which is more prevalent in Western countries, has a poor prognosis and inevitably requires multimodal treatment.[2]

It is well known that Tumor, Node, Metastasis (TNM) stage is one of the most reliable prognostic factors for gastric cancer,[3] but this is available only after surgery and requires careful histologic evaluation. In addition, there is often a discrepancy between preoperative clinical staging conducted using imaging and postoperative pathological staging.[4] To improve the outcome of GC patients, prognostic evaluation is important because it can affect the decision-making process regarding therapy, for example, regarding the extent of surgery or the duration of postoperative follow-up.

Increasing studies have shown that the dysregulation of cholesterol metabolism is associated with cancer development.[5,6] However the correlation of cholesterol in carcinogenicity can be cancer-type specific. While hypercholesteremia has positive correlations in breast and prostate cancers,[7,8] hypocholesterolemia has been reported to be associated with poor outcome in several types of cancer, including renal cell cancer, hepatocellular carcinoma, and lung cancer.[9–11] Also recent study prove that hypercholesterolemia was associated with better outcomes in immune check point-treated cancer patients.[12] However, there have been few studies of the relationship between serum TC concentration and prognosis in GC patients to date.

In this study, we analyzed a single large cohort of 1251 GC with surgically treated GC to characterize the prognostic role of

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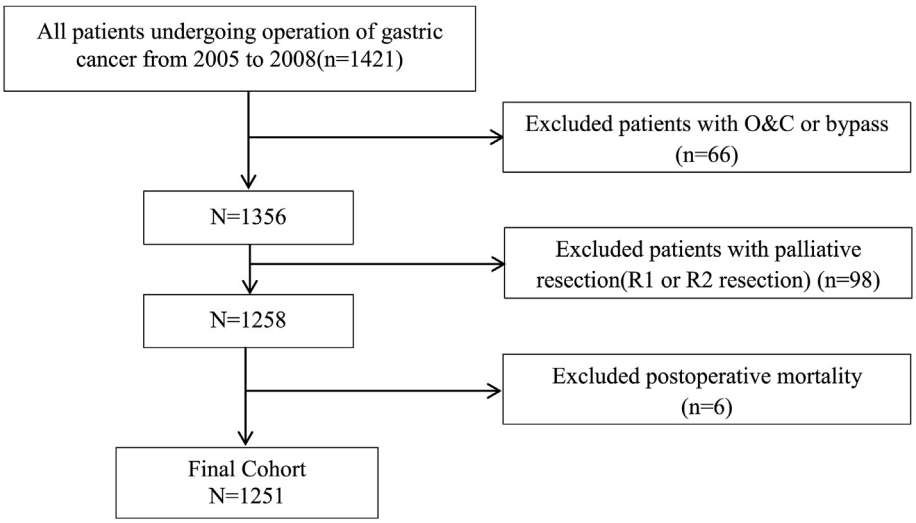

Fig. 1. Flow chart of inclusion and exclusion criteria for the full patient cohort.

preoperative serum TC after rigorous statistical balancing processes.

2. Methods

We retrospectively reviewed a database of 1421 patients who had undergone surgery for GC at Ajou University Hospital between January 1, 2005 and December 31, 2008. Of these, 170 patients who underwent Open and closure (O&C), bypass, and palliative resections with macro- or microscopically positive pathological margins were excluded. The remaining 1251 patients comprised the final cohort.

Data collected prospectively during the perioperative period (within 30 days of surgery) included age, sex, preoperative body mass index (BMI), the American Society of Anesthesiologists' (ASA) physical status classification system, surgical approach, tumor size, TNM stage, type of surgery, and 30 day postoperative complications (Clavien-Dindo classification, with $\geq$3 being considered a major complication).[13] Surgical resection and D1+/D2 lymphadenectomy were performed in accordance with the Japanese guidelines for treating GC.[14] Patient staging was adjusted according to the 7th edition of the American Joint Committee on Cancer Staging.[15]

Laboratory blood test data collected within the 3 weeks immediately preceding surgery included serum albumin, total protein, TC, and neutrophil, platelet, lymphocyte, and monocyte counts.

Our institution follows a standardized surveillance protocol and follows up patients for at least 5 years, with 3-monthly clinical assessment intervals for the first 2 years, followed by 6-monthly intervals for the three following years. After this, most patients undergo annual surveillance. The postoperative follow-up data that was collected included clinical assessment records, laboratory tests, radiological results, and endoscopic surveillance results. Computed tomography of the chest, abdomen, and pelvis are performed at least annually as the radiological surveillance imaging modality of choice in our institution. All-cause and cancer-specific mortality data were obtained from the Korean Cancer registry.

The outcomes we measure were, overall survival, recurrence free survival, and surgical outcomes, such as hospital stays, blood loss, type of complications and incidence of complications. Based on the previous studies, the relationship between serum TC level and mortality rate were U-shaped or L-shaped.[16] And serum TC level and the log hazard of deaths shows a L shaped relationship (Fig. 2).

Although categorization is not desirable from a statistical point of view, due to loss of information and power, patients were

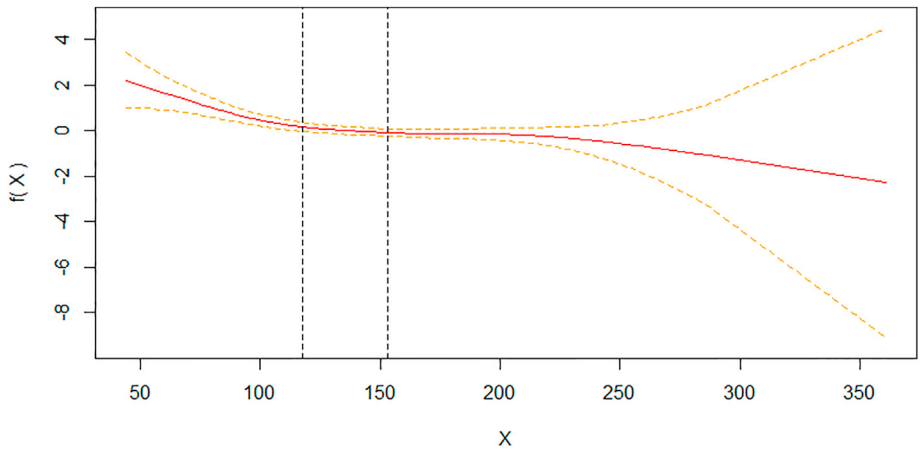

Fig. 2. L-Shape relationship of Total cholesterol on log hazard of death – dashed lines are optimal cut-off points, 117.6 mg/dl and 152.9 mg/dl respectively.

Table 1
Baseline characteristics between the low TC and high TC groups, before and after weighting.

	Unweighted study population					Weighted study population				
	Low TC [n = 224]	Normal TC [n = 396]	High TC [n = 631]	P value	SMD	Low TC [n = 224]	Normal TC [n = 159.59]	High TC [n = 129.54]	P value	SMD
Age (years)	58.53 (12.87)	58.73 (12.95)	56.74 (12.14)	**0.026**	0.105	58.53 (12.87)	59.49 (12.87)	59.99 (11.36)	0.525	0.079
Sex: Female	57 (25.4)	119 (30.1)	241 (38.2)	**0.001**	0.184	57.0 (25.4)	48.9 (30.7)	40.4 (31.2)	0.411	0.085
BMI (kg/m^2)	22.30 (3.00)	22.93 (3.09)	23.51 (3.18)	**<0.001**	0.261	22.30 (3.00)	22.35 (3.01)	22.42 (2.99)	0.935	0.027
ASA				**0.048**	0.15				0.536	0.161
1	116 (51.8)	203 (51.3)	342 (54.2)			116.0 (51.8)	85.0 (53.3)	56.3 (43.4)		
2	76 (33.9)	149 (37.6)	241 (38.2)			76.0 (33.9)	55.9 (35.1)	49.4 (38.1)		
≥3	32 (14.3)	44 (11.1)	48 (7.6)			32.0 (14.3)	18.6 (11.7)	23.9 (18.4)		
Extent of surgery				**0.009**	0.15				0.18	0.133
Total gastrectomy	65 (29.0)	77 (19.4)	126 (20.0)			159.0 (71.0)	123.3 (77.3)	103.0 (79.5)		
Subtotal gastrectomy	159 (71.0)	319 (80.6)	505 (80.0)			65.0 (29.0)	36.3 (22.7)	26.5 (20.5)		
Approach				**0.001**	0.21				0.29	0.095
MIS	53 (23.7)	139 (35.1)	240 (38.0)			53.0 (23.7)	47.8 (29.9)	30.9 (23.8)		
Open	171 (76.3)	257 (64.9)	391 (62.0)			171.0 (76.3)	111.8 (70.1)	98.7 (76.2)		
Histology				0.432	0.066				0.542	0.084
Differentiated	82 (36.6)	164 (41.4)	241 (38.2)			82.0 (36.6)	65.9 (41.3)	45.6 (35.2)		
Undifferentiated	142 (63.4)	232 (58.6)	390 (61.8)			142.0 (63.4)	93.7 (58.7)	83.9 (64.8)		
White Blood Cell ($x10^3/\mu L$)	10.85 (4.86)	9.81 (4.53)	8.36 (3.90)	**<0.001**	0.376	10.85 (4.86)	10.62 (4.49)	10.60 (4.86)	0.849	0.035
Neutrophil ($x10^3/\mu L$)	8803.04 (4888.90)	7588.99 (4726.54)	5570.45 (4010.07)	**<0.001**	0.479	8803.04 (4888.90)	8621.48 (4581.96)	8145.23 (5060.16)	0.578	0.09
Lymphocyte ($x10^3/\mu L$)	1330.33 (631.52)	1547.80 (700.11)	2041.19 (752.53)	**<0.001**	0.676	1330.33 (631.52)	1365.49 (638.86)	1576.90 (637.48)	**<0.001**	0.258
Monocyte ($x10^3/\mu L$)	733.71 (454.77)	636.69 (369.95)	511.01 (304.68)	**<0.001**	0.393	733.71 (454.77)	678.38 (366.84)	636.16 (388.22)	0.139	0.159
Hemoglobin (mg/dl)	11.50 (2.00)	12.27 (1.93)	13.26 (1.79)	**<0.001**	0.617	11.50 (2.00)	11.76 (2.00)	11.90 (1.90)	0.169	0.136
Platelet ($x10^3/\mu L$)	233.72 (94.53)	241.74 (77.22)	268.70 (74.50)	**<0.001**	0.286	233.72 (94.53)	238.46 (88.39)	245.16 (88.26)	0.594	0.084
Albumin (mg/dl)	3.25 (0.52)	3.70 (0.49)	4.12 (0.42)	**<0.001**	1.203	3.25 (0.52)	3.39 (0.47)	3.46 (0.50)	**0.002**	0.275
Extent of LN dissection				0.2	0.079				0.148	0.168
D1 +	121 (54.0)	220 (55.6)	378 (59.9)			121.0 (54.0)	77.8 (48.8)	79.3 (61.2)		
D2	103 (46.0)	176 (44.4)	253 (40.1)			103.0 (46.0)	81.8 (51.2)	50.2 (38.8)		

TC: total cholesterol; LG: laparoscopic gastrectomy; SMD: standardized mean difference; BMI: body mass index; ASA: American Society of Anesthesiologists Physical Status Classification System; MIS:minimal invasive surery; LN: lymph node.
Dichotomous variables are displayed as n (%), continuous parameters are displayed as mean (standard deviation (SD))

assigned to three groups according to serum TC concentration to compare the surgical outcomes. The "CatPredi" R package based on bootstrapping sampling, was used to identify optimal cut-off values, which were 117.6 mg/dl (c - index: 0.5818) and 152.9 mg/dl (c-index: 0.5648) respectively (Fig. 2).[17]

In order to balance the observed covariates, propensity score weighting technique, which was based on the machine learning technique, was applied with R package 'twang'.[18] For estimating the propensity score, and the following variables were included in the model: age, sex, BMI, ASA, type of surgical approach, extent of resection, extent of lymph node dissection, tumor size, adjuvant chemotherapy, and preoperative laboratory findings including CBC and differential counts, total protein and albumin.

To estimate the inverse probability of treatment weighted (IPTW)-adjusted hazard ratio (HR), double-robust (DR) Cox proportional hazard regression was performed. The level of the statistical significance threshold for all other tests were set as $P < 0.05$. All analyses were performed using R software version 3.6.0 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). The R packages "rms", "Hmisc", "twang", "CatPredi" and "survey" were applied.

3. Results

Of 1421 consecutive patients with a diagnosis of GC who underwent surgery, 66 patients underwent an O&C and bypass, 98 patients had palliative resection at the time of surgery, and there were six cases of postoperative mortality, all of whom were excluded. Exclusion of these patients resulted in a sample size of 1251 patients who had undergone curative elective surgery for GC (Fig. 1).

3.1. Patients characteristics and surgical outcomes

Table 1 lists the clinico-pathological characteristics of the entire cohort. We stratified the patients into 3 groups by using cutoff values of 117.6 mg/dl and 152.9 mg/dl. Of the 1251 patients, 224 had low serum TC (≤117.6 mg/dL: Low TC), 396 had normal TC (>117.6 mg/dL and ≤152.9: normal TC) and 631 had high TC (>152.9 mg/dL). In LTC group. There were considerable differences compared to other groups. Patients in LTC group were more likely to have a higher proportion of male, low BMI, larger tumor size, more advanced tumor stage, and more abnormal laboratory results, such as high white blood cell count, low hemoglobin, low platelet count, and low albumin. After IPTW adjustment with the propensity score, which is estimated using GBM, the standard mean difference for most preoperative covariates was <0.2, indicating that weighted population in the groups was comparable except for a few covariates such as lymphocyte and albumin.

Table 2 shows the postoperative outcomes and histopathological characteristics of the original cohorts. Even after rigorous balancing process, the severe complication rate (Clavien-dindo grade ≥ 3) was higher in Low TC group ($P = 0.004$).

The type and grade of complications are listed in Table 3. Both overall complication rate was higher in the low TC group (21.4% in low TC, 12.7% in normal TC, 4.7% in high TC) and severe complication rate (15.2% in low TC, 5.5% in normal TC, 4.7% in high TC) are higher in the low TC group even after balancing process ($P = 0.004$). In the low TC group, intra-abdominal bleeding, ileus, urinary complication and other complications are more frequent after weighted population ($P < 0.05$).

Table 2
Operative outcomes of the Low TC and High TC groups, before and after weighting.

	Unweighted study population					Weighted study population				
	Low TC [n = 224]	Normal TC [n = 396]	High TC [n = 631]	P value	SMD	Low TC [n = 224]	Normal TC [n = 159.59]	High TC [n = 129.54]	P value	SMD
Hospital stay (days)	15.90 (18.25)	11.73 (10.70)	11.33 (8.20)	**<0.001**	0.214	15.90 (18.25)	12.63 (14.59)	12.65 (9.54)	0.067	0.141
Tumor Size (cm)	5.65 (3.76)	4.30 (2.73)	4.01 (2.79)	**<0.001**	0.337	5.65 (3.76)	5.07 (3.45)	5.57 (4.01)	0.329	0.104
PRM (cm)	4.93 (3.54)	5.09 (3.26)	5.10 (4.54)	0.847	0.031	4.93 (3.54)	4.84 (3.14)	5.16 (3.40)	0.696	0.065
DRM (cm)	6.10 (4.34)	5.91 (4.18)	6.34 (4.32)	0.289	0.067	6.10 (4.34)	5.76 (4.25)	6.04 (4.19)	0.71	0.052
Lauren classification				0.344	0.122				0.882	0.11
Diffuse	99 (44.2)	151 (38.1)	267 (42.3)			99.0 (44.2)	62.7 (39.3)	47.5 (36.7)		
Intestinal	94 (42.0)	179 (45.2)	285 (45.2)			94.0 (42.0)	71.3 (44.7)	60.8 (46.9)		
Mixed	22 (9.8)	52 (13.1)	55 (8.7)			22.0 (9.8)	19.4 (12.2)	16.2 (12.5)		
undetermined	9 (4.0)	14 (3.5)	24 (3.8)			9.0 (4.0)	6.2 (3.9)	5.0 (3.8)		
Complications				**<0.001**	0.267				**0.004**	0.262
None	176 (78.6)	348 (87.9)	560 (88.7)			176.0 (78.6)	139.4 (87.4)	116.9 (90.2)		
CD Grade 1,2	14 (6.2)	27 (6.8)	47 (7.4)			14.0 (6.2)	11.5 (7.2)	6.6 (5.1)		
CD Grade 3,4	34 (15.2)	21 (5.3)	24 (3.8)			34.0 (15.2)	8.7 (5.5)	6.1 (4.7)		
pT-stage				**<0.001**	0.341				0.109	0.282
T1	85 (37.9)	204 (51.5)	367 (58.2)			85.0 (37.9)	72.9 (45.7)	51.3 (39.6)		
T2	31 (13.8)	54 (13.6)	80 (12.7)			31.0 (13.8)	25.2 (15.8)	18.9 (14.6)		
T3	40 (17.9)	59 (14.9)	106 (16.8)			40.0 (17.9)	24.6 (15.4)	36.1 (27.9)		
T4	68 (30.4)	79 (19.9)	78 (12.4)			68.0 (30.4)	36.8 (23.1)	23.2 (17.9)		
pN-stage				**<0.001**	0.261				0.755	0.139
N0	102 (45.5)	228 (57.6)	405 (64.2)			102.0 (45.5)	86.6 (54.2)	69.4 (53.6)		
N1	38 (17.0)	62 (15.7)	79 (12.5)			38.0 (17.0)	22.5 (14.1)	16.3 (12.6)		
N2	35 (15.6)	45 (11.4)	59 (9.4)			35.0 (15.6)	20.3 (12.7)	18.9 (14.6)		
N3	49 (21.9)	61 (15.4)	88 (13.9)			49.0 (21.9)	30.2 (18.9)	25.0 (19.3)		
Lymph node yield	39.34 (15.83)	36.12 (14.51)	35.01 (14.69)	**0.001**	0.19	39.34 (15.83)	37.96 (15.10)	36.49 (14.18)	0.31	0.126
Total number of positive lymph nodes	4.38 (7.44)	3.45 (7.95)	2.71 (6.34)	**0.008**	0.155	4.38 (7.44)	4.94 (10.80)	3.79 (7.19)	0.612	0.089
Adjuvant chemotherapy				**0.036**	0.125				0.104	0.165
Yes	70 (31.2)	91 (23.0)	146 (23.1)			154.0 (68.8)	118.2 (74.0)	103.0 (79.5)		
No	154 (68.8)	305 (77.0)	485 (76.9)			70.0 (31.2)	41.4 (26.0)	26.5 (20.5)		
Stage (AJCC, 7th Edition%)				**<0.001**	0.29				0.469	0.144
I	93 (41.5)	222 (56.1)	387 (61.3)			93.0 (41.5)	81.8 (51.3)	60.9 (47.0)		
II	47 (21.0)	81 (20.5)	113 (17.9)			47.0 (21.0)	32.5 (20.4)	25.9 (20.0)		
III	84 (37.5)	93 (23.5)	131 (20.8)			84.0 (37.5)	45.3 (28.4)	42.7 (33.0)		

TC: total cholesterol; PRM: proximal resection margin; DRM: distal resection margin; CD: Clavien-dindo; SMD: standardized mean difference.

Table 3
Postoperative complications of Low TC and High TC groups, before and after weighting.

	Unweighted study population					Weighted study population				
	Low TC [n = 224]	Normal TC [n = 396]	High TC [n = 631]	P value	SMD	Low TC [n = 224]	Normal TC [n = 159.59]	High TC [n = 129.54]	P value	SMD
Complications				**<0.001**	0.267				**0.004**	0.262
None	176 (78.6)	348 (87.9)	560 (88.7)			176.0 (78.6)	139.4 (87.4)	116.9 (90.2)		
CD Grade 1,2	14 (6.2)	27 (6.8)	47 (7.4)			14.0 (6.2)	11.5 (7.2)	6.6 (5.1)		
CD Grade 3,4	34 (15.2)	21 (5.3)	24 (3.8)			34.0 (15.2)	8.7 (5.5)	6.1 (4.7)		
Type of Complication										
Wound	7 (3.1)	7 (1.8)	27 (4.3)	0.088	0.099	7.0 (3.1)	2.4 (1.5)	2.1 (1.6)	0.226	0.073
Fluid collection or abscess	5 (2.2)	2 (0.5)	5 (0.8)	0.088	0.101	5.0 (2.2)	0.5 (0.3)	2.0 (1.5)	0.25	0.115
Intra-abdominal bleeding	11 (4.9)	12 (3.0)	7 (1.1)	**0.004**	0.152	11.0 (4.9)	6.3 (4.0)	0.5 (0.4)	**0.042**	0.192
Intra-luminal bleeding	7 (3.1)	7 (1.8)	8 (1.3)	0.192	0.085	7.0 (3.1)	3.5 (2.2)	1.6 (1.2)	0.392	0.087
Intestinal obstruction	3 (1.3)	3 (0.8)	4 (0.6)	0.592	0.048	3.0 (1.3)	1.2 (0.7)	0.2 (0.1)	0.167	0.1
Ileus	3 (1.3)	1 (0.3)	3 (0.5)	0.202	0.084	3.0 (1.3)	0.2 (0.1)	0.2 (0.1)	**0.003**	0.097
Anastomosis stenosis	1 (0.4)	0 (0.0)	1 (0.2)	0.409	0.068	1.0 (0.4)	0.0 (0.0)	1.3 (1.0)	0.488	0.102
Leakage	6 (2.7)	4 (1.0)	5 (0.8)	0.077	0.097	6.0 (2.7)	3.0 (1.9)	1.8 (1.4)	0.714	0.059
Pancreatitis	0 (0.0)	0 (0.0)	3 (0.5)	0.228	0.065	0.0 (0.0)	0.0 (0.0)	0.6 (0.5)	0.231	0.064
Pulmonary	7 (3.1)	14 (3.5)	8 (1.3)	0.043	0.099	7.0 (3.1)	6.7 (4.2)	2.8 (2.1)	0.67	0.08
Urinary	1 (0.4)	1 (0.3)	1 (0.2)	0.749	0.035	1.0 (0.4)	0.1 (0.1)	0.0 (0.0)	**0.038**	0.057
Renal	0 (0.0)	1 (0.3)	0 (0.0)	0.339	0.047	0.0 (0.0)	1.6 (1.0)	0.0 (0.0)	0.324	0.095
Hepatic	1 (0.4)	0 (0.0)	0 (0.0)	0.101	0.063	1.0 (0.4)	0.0 (0.0)	0.0 (0.0)	0.574	0.063
Others	8 (3.6)	5 (1.3)	6 (1.0)	**0.02**	0.119	8.0 (3.6)	1.2 (0.8)	0.5 (0.4)	**<0.001**	0.158

TC: total cholesterol; CD: Clavien-dindo; SMD: standardized mean difference.
Dichotomous variables are displayed as n (%)

3.2. Long-term outcomes

In analysis of all patients, the median follow-up period was 75.43 months (95% confidence interval [CI]: 70.8–74.9) and 193 patients (15.4%) died. The 5-year IPTW-adjusted rates of OS for the low TC, normal TC, and high TC groups were 75%, 77.4% and 91.5% respectively. IPTW-adjusted Kaplan–Meier curves (Fig. 3) suggested that low TC (categorical variable) wasn't associated with

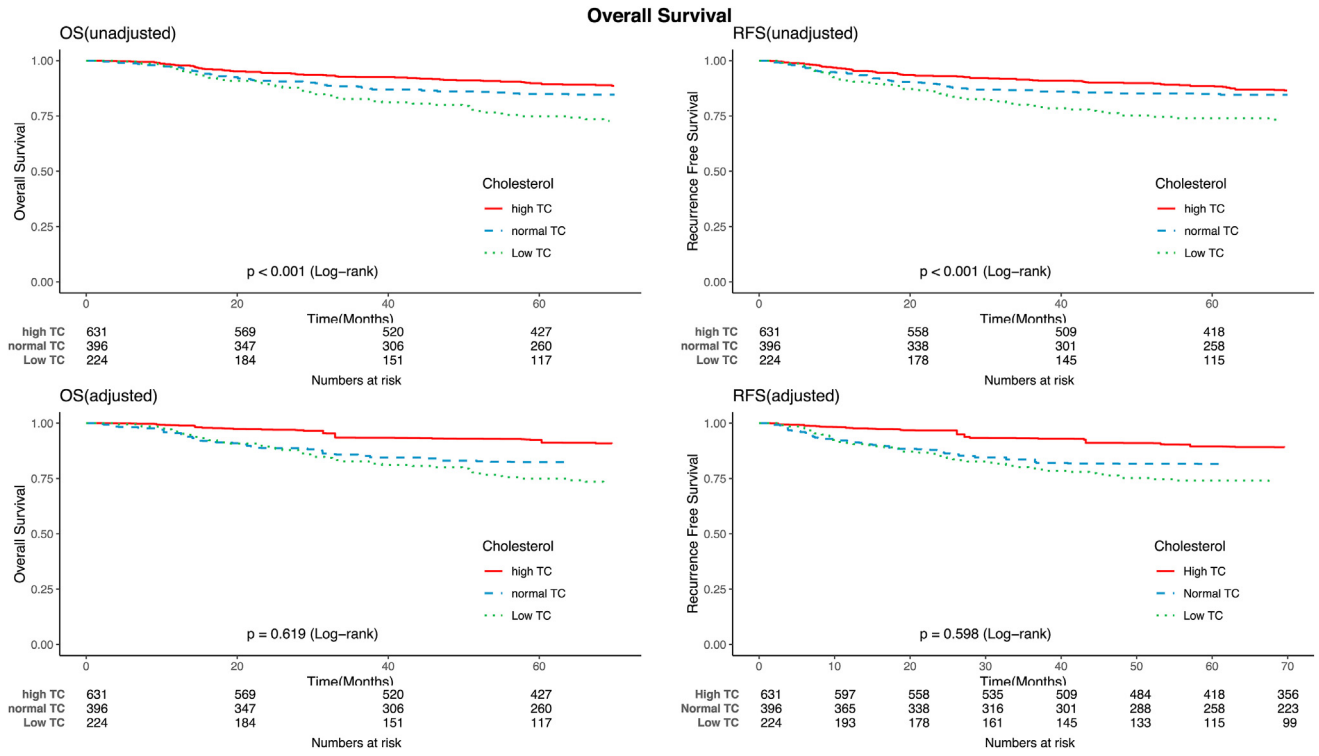

Fig. 3. Unadjusted and Adjusted Kaplan–Meier survival curves for mortality according to preoperative serum cholesterol concentration (Normal TC > 117.6 mg/dL and ≤ 152.9 mg/dl, Low TC ≤ 117.6 mg/dl, High TC > 152.9 mg/dl).

decreased OS or RFS in weighted population. (P > 0.05) To identify prognostic markers for the prediction of OS and RFS in the weighted cohort, 15 variables were evaluated using univariate Cox regression analysis, which showed that sex, BMI, tumor size, degree of tumor differentiation, surgical approach, total gastrectomy, development of complications, total cholesterol, use of adjuvant chemotherapy, and TNM stage had significant impacts on both OS and RFS. LN dissection was the only prognostic marker for OS in univariate analysis. (Supplement 1) After multivariable analysis of the entire cohort, only BMI, tumor size, total cholesterol (continuous variable), and TNM stage were identified as independent prognostic markers of OS and RFS. Degree of tumor differentiation and low TC (categorical variable) were significant only in OS. The HR for OS in total cholesterol (continuous variable; TC per 10 mg/dL) was 0.92 (95% CI 0.867–0.980; p = 0.009) and the HR for RFS was 0.93 (95% 0.873–0.988; p = 0.019) respectively (Table 4). The relative risk ratio of each predictor variable for overall survival is shown in Fig. 4, where the relative risk of serum TC 100 mg/dl versus 200 mg/dl is doubled. We build a nomogram for weighted-cox model (Fig. 5).

4. Discussion

We made following main findings in this study. First, preoperative serum TC concentration in GC patients was significantly correlated with advanced tumor progression and aggressive clinico-pathologic tumor characteristics. Second, even after rigorous adjustment of clinic-pathologic factors with PSW, preoperative serum TC (continuous variable) was an independent prognostic factor for both OS and RFS, in other words lowering TC is associated with shorter survival. Furthermore, low preoperative serum TC (<117.6 mg/dl) was a statistically significant risk factor affecting the occurrence of post-operative complications in GC patients. Thus, this study highlights important clinical implications of the preoperative serum TC concentration in patients with resectable GC: patients with a low serum TC concentration were more likely to have an unfavorable outcome.

Earlier epidemiologic studies had suggested that low serum TC is associated with a high incidence of several types of cancer and high mortality.[19–23] Two of these studies are of particular interest because they were population-based, prospective cohort studies. Casiglia et al reported a long duration, population-based prospective study that included 3257 patients,[19] which showed that patients of both genders in the lowest quintile of preoperative serum TC had the highest cancer-related and all-cause mortality. As expected, patients in the highest percentile group, who were all male, showed the highest cardiovascular mortality. In addition, the Japan Public Health Center-based Prospective Study (JPHC), which was also a population-based cohort study (n = 116,686), showed that low cholesterol was associated with a high incidence of gastric and liver cancer.[21] Also Increasing studies indicates low preoperative TC is not only associated with postoperative complications, but also with poor long-term outcomes.[24]

However, the exact mechanism by which the low level of serum cholesterol affects the survival of patients with GC is as yet unclear. Cholesterol is known to have an important role in the maintenance of cell membranes and to modulate membrane fluidity and function, including trans-membrane signaling and cell adhesion to the extracellular matrix. It is also known that proliferating cancer cells depend on either *de novo* synthesis of cholesterol in the endoplasmic reticulum or its uptake from the circulation by low-density lipoprotein (LDL) receptor-mediated endocytosis.[25] Caruso et al reported that diffuse types of GC preferentially meet their high cholesterol requirements by increasing endogenous cholesterol synthesis through upregulation of HMG-CoA reductase (3-hydroxy-3-methylglutaryl coenzyme A reductase), whereas intestinal type

Table 4
Multivariable analysis for OS and RFS of Weighted Population.

	Overall survival			Recurrence free survival		
Variables	HR	95% CI	P value	HR	95% CI	P value
Age (per year)	1.02	0.999–1.035	0.06	1.02	0.999–1.035	0.053
Sex: Male	1.49	0.859–2.592	0.154	1.33	0.787–2.256	0.285
BMI (kg/m^2)	0.91	0.858–0.973	**0.005***	0.93	0.879–0.994	**0.031***
ASA						
0-1	reference			reference		
2	1.16	0.726–1.865	0.528	1.01	0.629–1.623	0.963
≥3	1.04	0.535–2.014	0.912	0.97	0.497–1.892	0.928
Approach						
MIS	reference			reference		
Open	1.10	0.459–2.655	0.823	1.16	0.488–2.766	0.733
Postoperative morbidity						
None	reference			reference		
Mild	0.62	0.267–1.447	0.270	0.65	0.289–1.462	0.298
Severe	1.10	0.512–2.390	0.797	1.21	0.565–2.613	0.617
Size (cm)	1.08	1.017–1.156	**0.013***	1.08	1.007–1.149	**0.029***
Histology						
Differentiated	reference			reference		
Undifferentiated	1.73	1.124–2.675	**0.013***	1.74	1.125–2.705	**0.012***
Extent of surgery (total)						
Subtotal	reference			reference		
Total	1.28	0.803–2.028	0.301	1.25	0.792–1.970	0.338
Hemoglobin (mg/dl)	1.03	0.926–1.157	0.541	1.04	0.941–1.182	0.453
Albumin (mg/dl)	1.19	0.780–1.840	0.409	1.14	0.739–1.760	0.552
LN dissection						
D1+	reference			reference		
D2	1.08	0.734–1.595	0.689	1.02	0.680–1.650	0.920
Adjuvant CTx						
No	reference			reference		
Yes	1.01	0.664–1.545	0.952	1.09	0.693–1.727	0.655
T.cholesterol (continuous*)	0.92	0.867–0.980	**0.009***	0.93	0.873–0.988	**0.019***
T.cholesterol						
High TC	reference					
Normal TC	1.69	0.920–3.120	0.090	1.63	0.897–2.975	0.108
Low TC	1.70	1.004–2.868	**0.048***	1.52	0.909–2.569	0.109
pStage						
I	reference			reference		
II	3.35	1.314–8.542	**0.011***	3.85	1.692–12.651	**0.002***
III	11.61	5.300–25.45	**<0.001***	11.12	5.229–28.202	**<0.001***

OS: overall survival; RFS: recurrence free survival; CI: confidence interval; HR: hazard ratio; BMI: body mass index; ASA: American Society of Anesthesiologists Physical Status Classification System; MIS: minimal invasive surgery; LN: lymph node; CTx: chemotherapy; T.cholesterol: Total cholesterol; pStage: pathological stage by AJCC 7th classification, continuous*: per 10 mg/dL

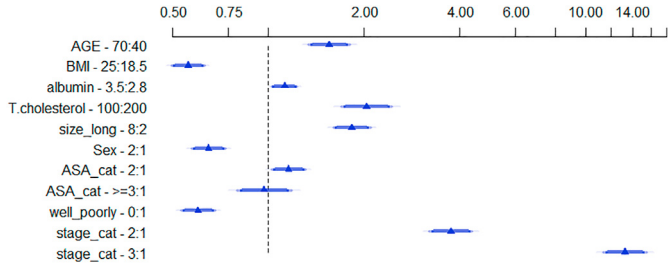

Fig. 4. Hazard ratios and multi-level confidence bars for effects of predictors.

GC cells meet these requirements by increasing LDL receptor expression, as well as by upregulating HMG-CoA reductase activity. Thus, low serum TC may reflect tumor progression in GC patients.[26]

Moreover, according to previous epidemiologic studies, low serum cholesterol is associated with a higher cancer incidence and greater cancer-related mortality. This might be explained by the relationship between the immune system and cholesterol metabolism. There have been several reports that low serum TC is associated with impairment of the immune system. Hypocholesterolemic men were found to have significantly fewer circulating lymphocytes, T cells, and CD8 cells than men with hypercholesterolemia.[27] Recently, Bensinger et al reported that cellular cholesterol enrichment is essential for the activation and proliferation of CD4+ T cells.[28] Cholesterol binds directly to the T cell receptor α-chain, regulating receptor nanoclustering and activation. Furthermore, T cell activation triggers simultaneous suppression of the liver X receptor pathway for cholesterol transport and induction of the sterol regulatory element-binding protein pathway for cholesterol synthesis. However, even after T cell activation, if cholesterol enrichment is not achieved, T cell proliferation is prevented. Therefore, a sustained low level of serum cholesterol may impair cell-mediated immunity and lead to immune escape and cancer progression. Thus, low serum cholesterol might be associated with tumorigenesis or tumor progression.

Chronic inflammation is known to be involved in the development and progression of GC. The tumor-associated microenvironment comprises tumor cells and associated stromal cells recruited by the tumor.[29] Various inflammatory mediators, such as cytokines (TNF-α, IL-6, IL-1β), chemokines (CC- and CXC- receptors), and matrix metalloproteinases establish an inflammatory network in the tumor-associated microenvironment.[30] In addition, the concentrations of these inflammatory mediators influence the prognosis of GC patients.[31] The serum concentrations of inflammatory mediators, such as interleukin-6, interleukin-10, and CRP, are reported to be higher in patients with hypocholesterolemia,[32] while a recent study demonstrated that low serum cholesterol is an

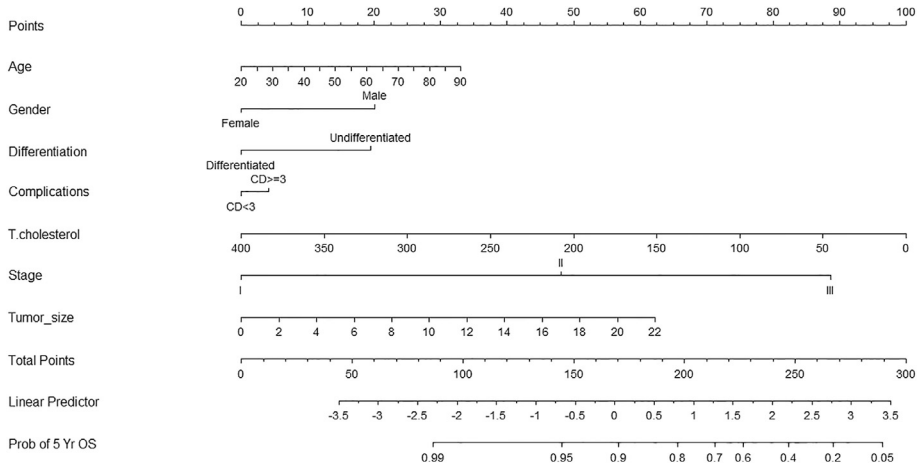

Fig. 5. Clinical nomogram for gastric cancer patients estimating the probability of surviving for 5 years.

independent prognostic factor for patients with severe sepsis.[33] These findings suggest that hypocholesterolemia is an epiphenomenon of the SIR. Thus, low serum cholesterol might reflect the level of cancer-related inflammation, and these hyper-catabolic and inflammatory response can exacerbate the tumor induced cachexia and hypocholesterolemia.

This study has several limitations. First, it was a retrospective study carried out in a single institution and may have selection and sampling bias. Even if we control the balance for measured factors with rigorous statistical correction, there may be unmeasured confounding factors, such as chronic liver disease, critical ill condition, which are also associated with low TC. Second, since our analysis is based on the retrospective data of the Asian population, this may also limit the generalizability of our findings. Third, we only analyzed the relationship between serum TC and GC prognosis because our routine preoperative laboratory screen did not include assessment of LDL-cholesterol or high-density lipoprotein-cholesterol concentrations. So there could be collider bias and paradoxical association between lowering TC and worse survival. Fourth, any changes in serum TC level during the follow-up period were not recorded. Finally, our cut-off values are 117.6 mg/dl and 152.9 mg/dl, which are lower than the level of TC suggested in previous study.[34] The level of TC may vary depending on ethical or geographic differences.[35] Thus, further large cohort studies and external validation study should be performed to establish the precise preoperative serum TC concentration that has the highest prognostic value in patients with GC.

In summary, preoperative serum TC concentration is an independent prognostic factor for patients with resectable GC, with lower concentrations being associated with a lower probability of survival. Also low preoperative serum TC was associated with advanced tumor progression and increased postoperative complications. Further prospective studies using more large cohorts are required for external validation.

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Conflicts of interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.asjsur.2020.08.014.

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