Associations of Serum Total Bilirubin with Survival Outcomes in Patients with Cancer Cachexia: A Retrospective, Multicenter Cohort Study

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Research

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Abstract

Background: Cancer cachexia is a systemic paraneoplastic phenomenon involving multiple organs, including the liver. Total bilirubin (TBIL) is an easily obtained blood biomarker that reflects liver homeostasis. This work evaluated the prognostic value of serum TBIL in patients with cancer cachexia.

Methods: This study included 2,282 patients from a multicenter research database who were diagnosed with cancer cachexia between June 2012 and December 2019. The hazard ratio (HR) for all-cause mortality was analyzed using Cox proportional hazards regression models. The association of serum TBIL with all-cause mortality was modeled with restricted cubic splines. The optimal cutoff value for TBIL was calculated with maximally selected rank statistics.

Results: Among the participants, there were 1,327 (58.2%) males and 955 (41.8%) females. The mean patient age was 60.4 ± 1.5 years. The 12-month all-cause mortality rate for patients with cancer cachexia was 29.5% (95% CI: 27.6%-31.3%), resulting in a rate of 209.58 events per 1000 patient-years. An inverted L-shaped association between TBIL and all-cause mortality was observed. The cutoff point for TBIL for the prediction of the time to mortality was $< 21.7 \mu\text{mol/L}$. A high TBIL level but not the direct bilirubin (DBIL) or indirect bilirubin (IBIL) level was identified as an independent prognostic factor (HR, 1.60, 95% CI, 1.32-1.93). For patients with digestive system tumors, a high serum TBIL level ($\geq 21.7 \mu\text{mol/L}$) was significantly associated with mortality. **Conclusion:** High TBIL levels are associated with increased all-cause mortality in patients and might be a promising prognostic indicator in patients with cancer cachexia.

1. Introduction

Cancer cachexia is a systemic syndrome involving progressive body weight and muscle reductions, systemic inflammation as well as adipose tissue wasting that seriously affects patient quality of life and shortens the lifespan^{1,2}. Cachexia affects 70% of cancer patients and accounts for at least 22% cancer-related death cases³. Currently, there is no available standard treatment to fully reverse cancer cachexia⁴.

Cachexia Experts have reached an agreement on the definition and classification of cancer cachexia¹. Cancer cachexia was divided into three stages: precachexia, cachexia, and refractory cachexia. Based on this model, Blum et al improved the classification system by further developing a four-group model that improve the accuracy of ability of discrimination⁵. Importantly, high heterogeneity in the prognosis of patients with cancer cachexia has been observed⁶. However, there is no effective method of assessing the prognosis of patients with different stages of cachexia according to these classification systems. Notably, prognostic biomarkers help not only identify patients with poor clinical outcomes but also stratify patients based on the optimal treatment or eligibility for clinical trials. Therefore, there exists a significant demand for easily determinable and readily available prognostic biomarkers to guide the assessment of life expectancy.

Cancer cachexia can affect and/or be influenced by many organs, such as the liver⁷. It has long been known that the liver is a major contributor to cancer cachexia due to the presence of systemic inflammation and hepatic metabolism alterations^{8,9}. Bilirubin (BLB), as the final product of heme degradation, is essential to reflect liver function. For many years, BLB has been regarded as an inauspicious sign of liver dysfunction with very limited physiological benefits¹⁰. Temme et al found high serum total bilirubin (TBIL) levels, albeit within the normal range, was associated with reduced cancer mortality, especially nonlung cancer mortality (P for trend < 0.02) among 5,460 men and 4,843 women during a 10-year follow-up¹¹. Consistent with this finding, recent studies showed that mildly elevated TBIL levels may be associated with reduced prevalences of cardiovascular diseases (CVDs), cancer, and metabolic syndrome^{12,13}. However, the highest serum levels of TBIL were found in patients with cardiac cachexia, and they were correlated with reduction in systolic right ventricle (RV) function and increased right atrial pressure¹⁴. Cancer cachexia is complex, with a multifactorial etiology, and the association between serum TBIL levels and mortality in patients with cancer cachexia remains unclear. Blood is easily accessible and obtaining a blood sample is a minimally invasive procedure, which makes blood biomarkers appealing for use in clinical practice. TBIL has great potential for the prediction of individual clinical outcomes and the stratification of patients into different prognostic subgroups.

Herein, we established the cutoff point for TBIL for the prediction of all-cause mortality in Chinese cancer patients with cachexia. In addition, a summary analysis of the clinical parameters of the patients was conducted to evaluate the potential associations of those parameters with the serum TBIL level. The aim of this study was to evaluate the potential prognostic value of the serum TBIL level in patients with cancer cachexia.

2. Patient And Methods

2.1 Study Participants

Briefly, this retrospective cohort study included 12,792 patients age 22 to 94 years who were diagnosed with malignant cancer based on pathology and were enrolled at more than 40 clinical centers throughout China from June 1, 2012, through December 31, 2019. Patients with multiple hospitalizations were regarded as one case. All patients provided written informed consent. No other special exclusion criteria were imposed except evidence of hemolytic disease and refusal to participate in the study. Patients with insufficient data concerning body mass index (BMI), blood test results, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30 Version 3.0) responses, and survival duration were excluded, leaving 2,282 patients with cancer cachexia for inclusion in the current analysis (Fig. 1). The average age of recipients was 60.4 ± 1.5 years with 41.8% (955/2282) of them were female. The study was approved by the institutional review board and complied with current ethics guidelines.

2.2 Data Collection and Definitions

Diagnoses of malignant cancers were based on histological evidence after operation or needle biopsy. Baseline information, including age, sex, BMI, comorbidities (diabetes mellitus, hypertension, and hepatobiliary disease), alcohol consumption, alcohol consumption, tea consumption, cancer types, TNM stage, treatment method, mid-arm circumference (MAC), hand-grip strength (HGS), anorexia, sarcopenia, patient-generated subjective global assessment (PG-SGA), EORTC QLQ-C30 and nutrition interventions, was recorded at the time of diagnosis. TNM staging was conducted using the 8th Union for International Cancer Control TNM staging system. All biochemical indicators, such as serum albumin concentration, TBIL, direct bilirubin (DBIL), indirect bilirubin (IBIL), alanine aminotransferase (AST), aspartate aminotransferase (ALT), white blood cell count, neutrophil count, lymphocyte count, red blood cell (RBC) count, and platelet count, were evaluated before treatment (surgery, chemotherapy, radiotherapy, and other treatment) at the baseline visit. The EORTC QLQ-C30 includes 30 items that are summarized in a global health status subscale (dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties), functional subscales (physical, role, social, emotional, and cognitive), and symptom subscales (fatigue, pain, and nausea and vomiting)¹⁵.

2.3 Definition of Cachexia

The international consensus criterion for cancer cachexia was weight loss more than 5% over the past 6 months (in the absence of simple starvation) or weight loss greater than 2% in individuals already underweight according to current BMI ($\text{BMI} < 20 \text{ kg/m}^2$) or skeletal muscle mass (sarcopenia). Skeletal muscle depletion was assessed based on mid-upper-arm muscle area according to anthropometry (men $< 32 \text{ cm}^2$, women $< 18 \text{ cm}^2$)¹. Cancer cachexia was diagnosed when any one of the criteria was satisfied.

2.4 Statistical Analyses

Overall survival (OS) was defined as the interval between the first assessment in the clinic until the date of death, or end of follow-up (September 31, 2019), or time of last contact, whichever came first. The Kolmogorov–Smirnov test was used to test the normality of continuous data. Normally distributed data are presented as the means $\pm$ SD or as the medians and interquartile ranges (IQRs) for data with skewed distribution. Serum TBIL was log-transformed before analysis. A Cox proportional hazards model was used for univariate and multivariate OS analyses. Hazard ratios (HRs) and their 95% confidence intervals (CIs) were reported. Correlations between TBIL and nutritional parameters were explored using pairwise Spearman's rank correlation coefficients. A possible nonlinear relationship between TBIL and HR was analyzed by restricted cubic spline regression. TBIL was categorized based on an optimal cutoff point of $21.7 \text{ }\mu\text{mol/L}$ obtained by maximizing the sensitivity and specificity. Survival endpoints were assessed by Kaplan-Meier survival curves and compared by the log-rank test. Separate Cox models were applied to each subgroup in stratified covariate subgroup analyses. The Wald test were used to tested for trends across the median value for each quartile. Interaction tests were conducted with cross-classified multilevel interaction terms in the multivariable models. Kaplan–Meier survival curves are shown for the subgroups with significant (P for interaction < 0.1) interaction terms. All the statistical analysis was performed using R software (version 4.0.2).

3. Results

3.1 Characteristics of Serum TBIL in Patients with Cancer Cachexia

A total of 2,282 patients of cancer-related cachexia were diagnosed among 12,279 participants. By the end of the study, (December 31, 2019), 1,245 (5%) death cases were identified. The serum TBIL levels were higher in liver cancer patients and lower in patients with nasopharyngeal cancer and colorectal cancer (CRC) (Fig. 2). No association between the serum TBIL concentration and tumor stage was observed (Supplementary Fig. 1). Further potential associations between serum TBIL levels and covariates in patients with cancer cachexia are presented in Supplementary Fig. 2. The serum TBIL levels were positively correlated with AST and ALT levels. Weak correlations were found between serum TBIL levels and other clinical indexes (age, BMI, albumin, platelet count, RBC count, HGS, Karnofsky Performance Status (KPS) score, EORTC QLQ-C30 score, and PG-SGA score).

3.2 Association of Serum TBIL with Overall Survival

The results of the univariate and multivariate Cox regression analyses of the associations of serum TBIL levels with OS in cancer cachexia patients are shown in Supplementary Table 1. Most baseline characteristics were associated with an increased risk of mortality in the univariate analyses. Age, tumor type, TNM stage, albumin, TBIL, neutrophil count, AST, ALT, RBC count, platelet count, HGS, sarcopenia, and EORTC QLQ-C30 score were independent prognostic factors in multivariate analysis. However, DBIL and IBIL were not independent prognostic markers. Restricted cubic spline models revealed that the risk of mortality was positive correlated with the serum TBIL level (Fig. 3). In addition, TBIL was associated with a poor prognosis in patients with cancer cachexia after adjustment for sex, age, tumor type, TNM stage, alcohol intake, albumin, neutrophil count, AST, ALT, RBC count, platelet count, HGS, sarcopenia, and EORTC QLQ-C30 score (Table 1). Each increase of one standard deviation of serum Tbil was significantly associated with a 13% increase in the risk of mortality in Model b (HR, 1.13; 95% CI, 1.08–1.18; $P < 0.001$). When the serum TBIL level was divided into tertiles, tertiles 2 (HR, 1.16; 95% CI, 1.04–1.39) and 3 (HR, 1.32; 95% CI, 1.14–1.54) were associated with a higher risk of mortality (P for trend < 0.001).

Table 1
The association between TBIL and hazard ratio of cachexia patients

TBIL	Model a		Model b	
	HR 95%CI	P-value	HR 95%CI	P-value
As continuous (per SD)	1.17 (1.13,1.22)	< 0.001	1.13 (1.08,1.18)	< 0.001
By TBIL, $\mu\text{mol/L}$ cut-off				
Low (<21.7)	ref		ref	
High (≥ 21.7)	1.93 (1.62,2.30)	<0.001	1.60 (1.32,1.93)	< 0.001
Tertile, $\mu\text{mol/L}$				
Q1 (<9.1)	ref		ref	
Q2 (9.1–13.5)	1.16 (1.04,1.39)	0.040	1.26 (1.08,1.48)	0.004
Q3 (≥ 13.5)	1.32 (1.14,1.54)	< 0.001	1.45 (1.24,1.70)	< 0.001
<i>p</i> for trend		< 0.001		< 0.001
Model a: adjusted by gender, age, BMI, and TNM stage;				
Model b: adjusted by gender, age, BMI, TNM stage, alcohol intake, tumor types, TNM stage, radiotherapy, chemotherapy, KPS, albumin, RBC, platelet, HGS, sarcopenia, neutrophils, AST, ALT, EORTC QLQ-C30 score.				

3.3 Demographics and Disease Characteristics Dichotomized by TBIL in Patients with Cancer Cachexia

Patients was further stratified according to the cutoff point of 21.7 $\mu\text{mol/L}$ that was the best for the prediction of OS (Supplementary Fig. 3A). Kaplan-Meier curves revealed that patients with high TBIL levels had poorer OS (Supplementary Fig. 3B). Table 2 presents the characteristics of 234 patients with cancer cachexia stratified by high and non-high prediagnosis levels of TBIL. Patients with high TBIL levels tended to be older and with higher percent of male, anorexia, hepatobiliary disease, and receive radiotherapy and chemotherapy. Higher TBIL accompanied by other biochemical criteria: higher neutrophil count, AST, ALT, and higher DBIL level, lower albumin, lymphocyte count, RBC count, and lower platelet count. Furthermore, a higher TBIL level was associated with a higher PG-SGA score, and EORTC QLQ-C30 score (Supplementary Table 2), lower KPS score and nutrition support. Although the distribution of TBIL levels differed among cancer populations, TBIL levels were significantly associated with the OS of patients with digestive system tumors, especially those with CRC. (data not shown) (Fig. 4).

Table 2
Baseline characteristic of the study cancer patients with cachexia stratified by TBIL.

Characteristic	TBIL low n = 2048	TBIL high n = 234	P-value
Population Characteristic			
Sex (%)			
Male	1153 (56.3%)	174 (74.4%)	< 0.001
Female	895 (43.7%)	60 (25.6%)	
Age (mean (SD))	58.67 (11.50)	60.88 (11.36)	0.005
BMI, kg/m ² , mean (SD)	20.9 (3.19)	20.74 (3.42)	0.483
Diabetes, yes, n (%)	159 (7.8%)	26 (11.1%)	0.099
Hypertension, yes, n (%)	324 (15.8%)	48 (20.5%)	0.081
Hepatobiliary disease, yes, n (%)	162 (7.9%)	33 (14.1%)	0.002
Smoke, yes, n (%)	927 (45.3%)	121 (51.7%)	0.071
Alcohol, yes, n (%)	476 (23.2%)	65 (27.8%)	0.143
Tea, yes, n (%)	538 (26.3%)	63 (26.9%)	0.891
Clinical Characteristic			
Lung cancer (%)	413 (20.2%)	34 (14.5%)	0.049
Liver cancer (%)	61 (3.0%)	35 (15.0%)	< 0.001
Gastric cancer (%)	458 (22.4%)	44 (18.8%)	0.245
Colorectal cancer (%)	502 (24.5%)	56 (23.9%)	0.908
Esophagus cancer (%)	184 (9.0%)	10 (4.3%)	0.02
Pancreatic cancer (%)	47 (2.3%)	21 (9.0%)	< 0.001
Gynecological and breast cancer (%)	256 (12.5%)	14 (6.0%)	0.005
Other cancer (%)	127 (6.2%)	20 (8.5%)	0.213

Data are represented as mean (SD), median (interquartile range), or number (%).

IQR, interquartile range; BMI, body mass index; KPS, Karnofsky Performance Status; TBIL, total bilirubin; DBIL, direct bilirubin; WBC, white blood cell; AST, alanine aminotransferase; ALT, aspartate aminotransferase; RBC, red blood cell count; MAC, mid-arm circumference; HGS, hand grip strength; PG-SGA, patient-generated subjective nutrition assessment.

For TBIL, low < 21.7μmol/L; high ≥ 21.7μmol/L

Characteristic	TBIL low n = 2048	TBIL high n = 234	P-value
TNM stage (%)			
I	176 (8.6%)	27 (11.5%)	0.083
II	429 (20.9%)	40 (17.1%)	
III	558 (27.2%)	53 (22.6%)	
IV	885 (43.2%)	114 (48.7%)	
Radiotherapy, yes, n (%)	1178 (57.5%)	84 (35.9%)	< 0.001
Chemotherapy, yes, n (%)	977 (47.7%)	93 (39.7%)	0.025
TBIL, $\mu\text{mol/L}$, median (IQR),)	10.96 (4.07)	60.06 (70.76)	< 0.001
DBIL, $\mu\text{mol/L}$, median (IQR),)	3.25 (2.11)	32.9 (52.15)	< 0.001
IBIL, $\mu\text{mol/L}$, median (IQR),)	7.70 (3.55)	27.16 (28.92)	< 0.001
Albumin (mean (SD))	37.48 (5.32)	35.57 (6.28)	< 0.001
WBC, $10^9/\text{L}$, mean (SD)	7.13 (8.44)	8.06 (3.70)	0.094
Neutrophil, $10^9/\text{L}$, mean (SD)	4.72 (3.17)	5.92 (3.21)	< 0.001
Lymphocyte, $10^9/\text{L}$, mean (SD)	1.5 (0.71)	1.38 (1.13)	0.031
AST (mean (SD))	25.57 (20.89)	85.57 (271.77)	< 0.001
ALT (mean (SD))	24.85 (27.32)	76.08 (173.9)	< 0.001
RBC (mean (SD))	4.07 (0.65)	3.96 (0.78)	0.013
Platelet (mean (SD))	246.5 (100.39)	212.59 (104.7)	< 0.001
KPS (mean (SD))	83.42 (14.67)	76.37 (20.32)	< 0.001
MAC, cm, mean (SD)	25.1 (3.50)	24.65 (3.90)	0.068
HGS, kg, mean (SD)	23.34 (9.90)	22.57 (9.98)	0.263
Anorexia, yes, n (%)	508 (24.8%)	90 (38.5%)	< 0.001

Data are represented as mean (SD), median (interquartile range), or number (%).

IQR, interquartile range; BMI, body mass index; KPS, Karnofsky Performance Status; TBIL, total bilirubin; DBIL, direct bilirubin; WBC, white blood cell; AST, alanine aminotransferase; ALT, aspartate aminotransferase; RBC, red blood cell count; MAC, mid-arm circumference; HGS, hand grip strength; PG-SGA, patient-generated subjective nutrition assessment.

For TBIL, low < 21.7 $\mu\text{mol/L}$; high $\geq$ 21.7 $\mu\text{mol/L}$

Characteristic	TBIL low n = 2048	TBIL high n = 234	P-value
Sarcopenia, yes, n (%)	1518 (74.1%)	165 (70.5%)	0.267
PG-SGA, mean (SD)	9.31 (4.44)	11.53 (4.73)	< 0.001
EORTC QLQ-C30, mean (SD)	52.06 (10.85)	58.3 (13.68)	< 0.001
Parenteral nutrition, yes, n (%)	335 (16.4%)	83 (35.5%)	< 0.001
Enteral nutrition, yes, n (%)	474 (23.1%)	90 (38.5%)	< 0.001
Data are represented as mean (SD), median (interquartile range), or number (%).			
IQR, interquartile range; BMI, body mass index; KPS, Karnofsky Performance Status; TBIL, total bilirubin; DBIL, direct bilirubin; WBC, white blood cell; AST, alanine aminotransferase; ALT, aspartate aminotransferase; RBC, red blood cell count; MAC, mid-arm circumference; HGS, hand grip strength; PG-SGA, patient-generated subjective nutrition assessment.			
For TBIL, low < 21.7 μmol/L; high ≥ 21.7 μmol/L			

3.4 Subgroup Analyses of Effect Modifiers of the Association of TBIL with Survival

Stratified analyses were conducted to compare the relationship between TBIL and OS in several subgroups (Fig. 4 and Supplementary Table 3). Evidence of the association of a high TBIL level with an increased mortality risk was observed in the majority of subgroups. However, the similar associations were not found among patients over 65 years old with abnormal BMI values, TNM stage III disease, lung cancer, radiotherapy, and abnormal neutrophil counts. Interestingly, interactions were observed between high TBIL levels and BMI, TNM stage, and tumor type (all *P* for interaction < 0.1). There were no other moderating effects of variables on the association between TBIL (< 27.1 μmol/L vs ≥ 27.1 μmol/L) and all-cause mortality. Moreover, the differential effects of each variable were assessed (Supplementary Table 4). Three covariates, namely, low BMI, advanced tumor stage, and lung cancer, combined with elevated TBIL were associated with the worst prognosis in patients with cancer cachexia (Supplementary Fig. 4).

3.5 Sensitivity analysis

To test the robustness of our results, a sensitivity analysis was conducted by examining whether the association would change if individuals who died less than 6 months after the diagnosis of cancer cachexia or patients with hepatobiliary disease and hepatobiliary tumors were removed at baseline (Table 3). The results remained unchanged (adjusted HR, 1.44; 95% CI, 1.1–1.88 for excluding patients dying within 6 months, adjusted HR, 1.52; 95% CI, 1.23–1.88 for patients with hepatobiliary disease and hepatobiliary tumors).

Table 3

Hazard risk for all-cause mortality in cachexia patients with low TBIL by excluding patients dying within 6 months or patients with hepatobiliary disease and hepatobiliary tumor burden

TBIL	HR 95%CI	P-value	HR 95%CI	P-value
Sensitive analysis	Excluding patients dying within 6 months		Without hepatobiliary disease and hepatobiliary tumor burden	
As continuous (per SD)	1.12 (1.04,1.20)	0.002	1.13 (1.06,1.22)	< 0.001
By TBIL ($\mu\text{mol/L}$) cut-off				
Low (<21.7)	Ref.		Ref.	
High (≥ 21.7)	1.44 (1.10,1.88)	0.007	1.52 (1.23,1.88)	< 0.001
Tertile, $\mu\text{mol/L}$				
Q1 (<9.1)	Ref.		Ref.	
Q2 (9.1–13.5)	1.24 (1.08,1.51)	0.031	1.25 (1.06,1.47)	0.009
Q3 (≥ 15.2)	1.32 (1.08,1.62)	0.008	1.42 (1.20,1.68)	< 0.001
Adjusted by gender, age, BMI, TNM stage, alcohol consumption, tumor types, TNM stage, radiotherapy, chemotherapy, KPS, albumin, RBC, platelet, HGS, sarcopenia, neutrophils, AST, ALT, EORTC QLQ-C30 score.				

4. Discussion

To our knowledge, this study is the largest to directly evaluate the association of serum TBIL with all-cause mortality in patients with cachexia. The distribution of TBIL concentration was associated with several types of tumors in this cohort. Furthermore, TBIL was an independent prognostic marker in multivariate analysis. We then calculated the TBIL cutoff point for the prediction of mortality in Chinese cancer patients with cachexia. Using this cutoff point, we found that a high TBIL level was strongly associated with all-cause mortality. Further analysis showed that the mortality risk was especially elevated in patients with high TBIL levels who had low BMI values, advanced cancer stages, and digestive system cancers. In the sensitivity analysis, the exclusion of patients with evidence of hepatobiliary disease and hepatobiliary tumors did not change the positive association of TBIL with the risk of all-cause mortality. In general, serum TBIL can serve as an indicator for the daily assessment of the prognosis of patients with cancer cachexia.

Cancer cachexia is a multi-organ syndrome that involves more than just skeletal muscle pathogenesis^{7,16}. Hepatic function can directly or indirectly cause whole-body energy expenditure and increased mortality of patients with cancer cachexia^{17,18}. BLB (TBIL, DBIL, and IBIL), albumin, ALT, and AST are frequently used as indicators of liver function. Elevated physiological marker levels reflect liver

disease and hepatocyte injury. Similar to previous studies^{19–21}, these liver parameters, except DBIL and IBIL, were independent prognostic factors in this population-based cohort study. However, there was no association between serum TBIL and all-cause mortality in the subgroup of patients with hepatocellular carcinoma (HCC) and cachexia (HR, 1.40; 95% CI, 0.68–2.87, $P=0.641$, data not shown). It is conceivable that this is attributable to the complexity of pathological changes in the liver, and other mechanisms should be investigated. For example, the different effects of the various forms (alpha- to delta-) of serum TBIL should be explored. Another explanation might be the limited number of HCC participants in our cohort.

To date, a few studies have investigated the associations of BLB with other cancer risks. Moderately elevated serum BLB levels were found to be associated with a markedly reduced prevalence of CRC²². Meanwhile, two previous studies reported that serum TBIL was inversely correlated with lung cancer mortality. One study proposed that relatively higher levels of BLB may protect people against lung cancers²³. Laura et al also identified an association between elevated levels of serum BLB due to genetic factors and lower rates of lung cancer²⁴. Reports concerning breast cancer are consistent with these findings^{25,26}. Unfortunately, several studies have reported conflicting results regarding the relationship between the BLB concentration and cancer. Two cohort studies confirmed the absence of an association between BLB and CRC^{27,28}. This may be due to the fact that BLB has antioxidant and antineoplastic effects²⁹, and a number of studies have demonstrated that BLB inhibits the activity of cytotoxic T lymphocytes and promotes T regulatory cell (T_{reg}) expansion^{30,31}. Overall, consistent evidence that plasma BLB has a protective effect against cancer is lacking. In our study, in patients with digestive system tumors, there was a significant negative correlation between the TBIL level and overall mortality. We conjecture that BLB signaling to the gut microbiota and its regulation of the liver–gut axis may have led to the above results. This emphasizes the importance of further research to elucidate the current results and confirm whether TBIL can be used to predict survival in patients with cachexia. Further investigation of the genetic factors affecting BLB is also warranted. UDP-glucuronosyltransferase (UGT1A1) is a key enzyme involved in the metabolism of BLB, and its mutations cause BLB metabolism disorders, such as Gilbert's syndrome^{32,33}. The clinical impacts of UGT1A1 promoter gene variation have been thoroughly studied in CRC patients³⁴. UGT1A1*28 allele carrier status was found to be inversely associated with the development of CRC in males. Nevertheless, the limitation in most analyses was the lack of BLB measurements. In the future study, we will focus on combining genetic analyses with BLB analyses to further elucidate the mechanism.

Patients who are malnourished have a poor response to antitumor therapy and an elevated risk of cachexia-related mortality³⁵. Our interaction analyses established an association between TBIL and BMI, which is a nutrition status indicator. In the subgroup analysis, TBIL was an effective indicator of prognosis in patients with normal BMI values. Patients with high TBIL levels and low BMI values had shorter survival durations, according to the KM analysis. The mechanisms underlying this association are unclear. Interestingly, patients with high BMI tended to have a better prognosis, indicating that obesity

might have a protective role against cancer cachexia. Consistent with our study, several meta-analyses reported that women with higher BMI values had a lower lung cancer risk and better prognosis^{36,37}.

Some limitations of our study need to be considered. Serum TBIL concentrations change dynamically in healthy people, especially depending on the fasting state. The changes in TBIL in patients with cancer cachexia should be taken into consideration. Follow-up studies are warranted to detect the impacts of dynamic changes in TBIL on mortality in patients with cancer cachexia. Another limitation is that some unmeasured indicators, such as alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), which also reflect liver function, or other measured confounding factors may have affected our results.

5. Conclusion

In conclusion, we established for the first time the cutoff point of TBIL for the prediction of mortality in patients with cancer cachexia. A lower level of serum TBIL was associated with a decreased risk of all-cause mortality. This is important, as serum TBIL is an easily assessable, highly reproducible, and inexpensive biomarker and might be a promising clinical tool to find patients with cancer cachexia at risk of mortality. Long-term studies on the development and progression of cancer cachexia are needed to evaluate the causal associations between serum TBIL levels and cancer cachexia. A comprehensive understanding of these mechanisms may guide the selection of appropriate clinical treatments.

6. Abbreviations

AST

aspartate transaminase

ALT

alanine transaminase

BMI

body mass index

CI

confidence interval

DBIL

direct bilirubin

EORTC QLQ-C30

European Organization for Research and Treatment of Cancer QLQ-C30

GLIM

Global Leadership Initiative on Malnutrition

HGS

hand grip strength

HR

hazard ratio

IBIL

indirect bilirubin
MAC
mid-arm circumference
OS
overall survival
PG-SGA
patient-generated subjective nutrition assessment
RBC
red blood cell
SMI
skeletal muscle index
TBIL
total bilirubin
WBC
white blood cell

7. Declarations

Ethics approval and consent to participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Prior to data collection, all study participants signed an informed consent form. This study was approved by the institutional review board of Beijing Shijitan Hospital (ChiCTR1800020329). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional review board of Beijing Shijitan Hospital (ChiCTR1800020329) and individual consent for this retrospective analysis was waived. The authors acquired written informed consent from the patient.

Consent for publication

Consent for publication was obtained from all co-author.

Availability of supporting data

Data will be made available after request application and approval.

Competing interests

The authors have no conflicts of interest to declare.

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Authors' contributions

(I) Conception and design: Han-Ping Shi; (II) Administrative support: Han-Ping Shi; (III) Provision of study material or patients: All authors; (IV) Collection and assembly of data: Qi Zhang, Xi Zhang, Guo-Tian Ruan; (V) Data analysis and interpretation: Xiang-Rui Li, MD, Kang-Ping Zhang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors

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Figures

Figure 1

Diagram of participants included in the analysis

Figure 2

TBIL (log transformation) in different cancer types stratified by whether patients had cachexia

The spline was adjusted by sex, age, BMI, tumor type, TNM stage, radiotherapy, chemotherapy, KPS, albumin, TBIL, RBC count, platelet count, sarcopenia, HGS, anorexia, and EORTC QLQ-C30 score.

Figure 3

Association between TBIL (continuous) and overall survival.

The model was adjusted by sex, age, BMI, TNM stage, tumor type, TNM stage, radiotherapy, chemotherapy, KPS, albumin, RBC count, platelet count, HGS, sarcopenia, neutrophil count, and EORTC QLQ-C30 score, as appropriate.

Figure 4

Associations between TBIL stratified by the cutoff point as low (<21.7 μmol/L) and high (≥21.7 μmol/L) and overall survival in various subgroups

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