Title page

Title: The Glasgow Prognostic Score accurately predicts survival in patients with biliary tract cancer not indicated for surgical resection

Running title: GPS in patients with biliary tract cancer.

Key words: Glasgow Prognostic Score; biliary tract cancer; prognosis

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Abstract

The Glasgow Prognostic Score (GPS) and neutrophil to lymphocyte ratio (NLR) are associated with the survival in patients with various types of malignancy. The aim of this study was to investigate the prognostic value of the GPS and NLR in patients with biliary tract cancer (BTC) undergoing palliative chemotherapy or best supportive care (BSC). Fifty-two patients with newly diagnosed BTC were retrospectively evaluated. We investigated the correlation between the GPS, NLR, and the overall survival rates. The area under the receiver operating characteristics curve (AUC) was calculated to compare the predictive ability of each score. Both the univariate and multivariate analyses were performed to identify clinicopathological variables associated with the overall survival. There were significant differences between the GPS groups regarding the neutrophil levels ($P<0.0001$), Hb ($P=0.024$), Alb ($P<0.0001$) and CRP ($P<0.0001$). A significant difference in the overall survival was found between the groups stratified based on the GPS, NLR ($P<0.001$). The GPS had a higher AUC value (0.905) in comparison to the NLR (0.648). In the multivariate analysis, the sex ($P=0.002$), CA19-9 ($P<0.0001$) and the GPS ($P<0.0001$) were found to be

independently associated with the overall survival. Our results demonstrate that the GPS is an independent marker of the prognosis in patients with BTC undergoing palliative chemotherapy or BSC, and is superior to the NLR in terms of its prognostic ability.

Keywords: Biliary tract cancer, Glasgow Prognostic Score, Neutrophil to Lymphocyte Ratio, Prognosis

Introduction

Biliary tract cancer (BTC) is a malignant neoplasm originating from biliary epithelial cells [1, 2], and is classified based on the anatomical location, as follows: (1) intrahepatic cholangiocarcinoma (iCCA), (2) extrahepatic cholangiocarcinoma (eCCA), (3) gallbladder cancer (GBCA) and (4) periampullary cancer [3, 4].

The incidence and mortality of BTC are increasing worldwide [1, 5]. In Japan, BTC is the sixth leading cause of cancer-related death; more than 17,000 patients die from this disease annually [6]. In spite of the significant advances in the treatment, the mortality rate of BTC remains high, because it is usually diagnosed at an advanced stage, and median overall survival (OS) in patients with unresectable or metastatic BTC is less than one year following the diagnosis [1, 7, 8]. Therefore, a reliable predictor of survival is needed to allow for optimal treatment choices to be made to ensure a better patient outcome.

A systemic inflammatory response has been reported to be associated with poor survival in patients with various types of malignancy. Accumulating evidence indicates that the Glasgow Prognostic Score (GPS), based on

inflammation criteria and including only the serum C-reactive protein (CRP) and albumin levels, or the neutrophil to lymphocyte ratio (NLR), is a reliable and practical scoring system for outcome prognostication in patients with various types of malignancy, including hepatocellular carcinoma (HCC) [9, 10, 11].

With regard to BTC, recent reports have demonstrated that an elevated NLR was associated with poor outcomes in patients with iCCA or hilar cholangiocarcinoma undergoing surgical resection [12]. Oshiro et al. have also demonstrated that an elevated GPS was associated with poor outcomes in patients with eCCA undergoing surgical resection [13]. However, the patients enrolled in their studies included only those undergoing surgical resection. Therefore, it has not yet been determined whether the GPS or NLR is useful for predicting the outcomes in patients with BTC not indicated for surgical resection.

Therefore, in the present study, we investigated the prognostic value of these inflammation-based prognostic scores (the GPS, modified GPS and NLR) in patients with BTC undergoing either palliative chemotherapy or BSC.

Methods

Patients

Sixty-four patients with newly diagnosed biliary tract cancer who were treated in the Department of Gastroenterology and Hepatology, The Jikei University Daisan Hospital, between January 2005 and July 2013 were enrolled. All medical records were reviewed retrospectively. Eight patients were lost to follow-up. Four patients who showed clinical evidence of cholangitis were excluded according to the Tokyo guidelines [14, 15]. Patients who showed other inflammatory conditions were also excluded. In total, 52 patients with BTC were finally included and evaluated.

The diagnosis of BTC was confirmed either pathologically or by using images obtained from ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) or endoscopic retrograde cholangiopancreatography (ERCP) [16]. Tumor-related variables, such as the primary tumor site (intrahepatic bile duct, extrahepatic bile duct, gallbladder), and the extent of the disease (locally advanced or metastatic) were evaluated by these imaging techniques.

This study complied with the standards of the Declaration of Helsinki and the current ethical guidelines, and was approved by the institutional ethics board. Written informed consent for participation in the study was not obtained from patients, because this study did not report on a clinical trial, and the data were retrospective in nature and analyzed anonymously.

Inflammation-based prognostic scores and other variables

Blood samples were obtained before the initial treatment to measure the CRP, albumin and total bilirubin levels, alkaline phosphatase (ALP) as well as the white blood cell count (WBC), neutrophil, lymphocyte and platelet (Plt) counts, hemoglobin level (Hb), carcinoembryonic antigen (CEA) level and carbohydrate antigen 19-9 (CA19-9) level, as reported previously [17, 18].

The GPS was described previously. Briefly, patients with both an elevated CRP level (>1.0mg/dl) and hypoalbuminemia (<3.5g/dl) were allocated a score of 2, patients with only one of these biochemical abnormalities were allocated a score of 1 and patients with neither of these abnormalities were allocated a score of 0 [19]. The modified GPS (mGPS) was also calculated with the CRP and albumin values as follows: patients with both an elevated

CRP level (>1.0mg/dl) and hypoalbuminemia (<3.5g/dl) were allocated a score of 2, patients with an elevated CRP level (>1.0mg/dl) only were allocated a score of 1 and patients with a normal CRP level (≤ 1.0mg/dl) and any albumin concentration were allocated a score of 0 [20]. The NLR was calculated by dividing the neutrophil count by the lymphocyte count [9]. We used the median level (4.0) as the cut - off value for NLR.

Treatment and patient' follow-up

The decision to classify a patient as not suitable for surgical resection was made based on patient-related factors (medically unfit or unable to tolerate a major operation) and tumor-related factors (distant metastasis, tumor extension to more than the third branch of the remnant biliary tract, major invasion of the main portal vein or hepatic artery, and/or small estimated remnant liver volume) [21]. The patients were treated with palliative chemotherapy (tegafur, gimeracil and oteracil (TS-1) alone, gemcitabine alone or gemcitabine and TS-1 in combination) or best supportive care. The patients with obstructive jaundice underwent endoscopic retrograde biliary drainage or percutaneous transhepatic biliary drainage before commencement of the initial treatment.

The patients were followed carefully after the initial treatment by imaging techniques and analyses of the tumor marker levels. For patients who showed tumor progression, palliative chemotherapy or best supportive care was provided. The start date of the follow-up was the date of the initial diagnosis of BTC. The end of the follow-up was the time of the last follow-up (July 2013) or death.

Statistical analysis

Continuous variables are presented as the medians and ranges. For NLR, the median was used as the cut-off point for dividing the patients into subgroups. Categorical variables are presented as the numbers and percentages. Comparisons between the groups were made using the Kruskal-Wallis test for continuous and ordinal variables and the chi-square test for categorical variables. The overall survival rates were calculated using the Kaplan-Meier method, and differences in the survival rates between two groups were compared with the log-rank test. To assess the prognostic factors, both the univariate and multivariate analysis were performed using the Cox-proportional hazard model. Variables that proved to be significant in the univariate analysis were tested subsequently with a

multivariate Cox-proportional hazard model. To evaluate the discriminatory ability of each score, receiver operating characteristics (ROC) curves were generated, and the areas under the curve (AUC) were measured. A P-value <0.05 was considered to be significant. All statistical analyses were performed using the IBM SPSS Statistics software program, v.19.0 (IBM SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

The baseline characteristics of the patients are shown in Table 1. The median age of the patients was 79 (range 52-96) years. In this study, 31 (59.6%) patients were male and 21 (40.4%) patients were female. The primary tumor sites were the intrahepatic bile duct (16 (30.8%) patients), the extrahepatic bile duct (19 (36.5%) patients) and the gallbladder (17 (32.7%) patients). The diagnosis of BTC was confirmed histologically in 28 (53.8%) patients and made using imaging techniques in the remaining patients. Thirty-three (63.5%) patients with obstructive jaundice underwent endoscopic retrograde biliary drainage and 4 (7.7%) patients with obstructive

jaundice underwent percutaneous transhepatic biliary drainage. Palliative chemotherapy was administered in 21 (40.4%) patients (TS-1 monotherapy, 6 (11.5%) patients; gemcitabine monotherapy, 6 (11.5%) patients; combination of TS-1 and gemcitabine, 4 (7.7%) patients and other treatments, 5 (9.7%) patients). The remaining 31 (59.6%) patients received BSC.

Twenty-nine (55.8%) patients showed an elevated CRP level (>1.0g/dl) and 35 (67.3%) patients had hypoalbuminemia (<3.5g/dl). Thirteen (25%) patients were allocated a GPS of 0, 14 (26.9%) patients were allocated a GPS of 1 and 25 (48.1%) a GPS of 2, respectively. Twenty-six (50%) patients showed an elevated NLR (> 4) level.

The baseline characteristics of the patients grouped according to the GPS allocation are shown in Table 2. There were significant differences between the GPS groups in the neutrophils (P<0.0001), Hb (P=0.024), Alb (P<0.0001) and CRP (P<0.0001).

Comparison of the prognostic ability for overall survival

The median follow-up was 4 (range 1-71) months. At the end of the follow-up period, 8 (15.4%) patients were alive and 44 (84.6%) patients had died. The one-, three- and five- year overall survival rates were 16.5%, 14.1%

and 7.1%, respectively. The comparison of the overall survival according to the GPS, mGPS and NLR is shown in Figures 1- 3. A significant difference in overall survival was found across all scores (P<0.001 for all scores).

To assess the discriminatory ability of the GPS, mGPS and NLR, ROC curves for the survival status were constructed, and the AUC values were compared (Table 3, Figure 4). The GPS had a higher AUC value (0.905) in comparison to the mGPS (0.83) and NLR (0.648).

Prognostic factors

In the univariate analysis, the patient age (P=0.039), sex (P=0.01), neutrophil (P=0.045), Alb (P=0.001), CRP (P=0.019), the GPS (P<0.0001), the mGPS (P<0.0001), and the NLR (P=0.01) were associated with the overall survival (Table 4).

Due to the correlation between the CRP, albumin and GPS, mGPS, the variables (age, sex, GPS and NLR) were tested in a multivariate analysis. The multivariate analysis revealed that only the sex (HR 0.299, P=0.002), CA19-9 (HR 1.0, P<0.0001) and the GPS (HR 2.686, P<0.0001) were independently associated with the overall survival (Table 4).

Discussion

In the present study, we demonstrated that the GPS, an inflammation-based prognostic score, is an independent marker of the prognosis in patients with BTC undergoing palliative chemotherapy or BSC, and is superior to the mGPS and NLR in terms of prognostic ability.

There is increasing evidence that the host inflammatory response plays an important role in the development and progression of cancer. In the last few decades, the systemic inflammatory response, particularly that evidenced by an elevated CRP level, has been reported to play an important role in the progression of a variety of common solid tumors [22].

Several possible mechanisms have been proposed for CRP elevation in cancer patients. First, cancer cell growth could induce a tissue inflammatory response and thus increase the CRP level. Second, CRP might reflect an inflammatory response activated as a secondary process due to tumor necrosis or local tissue damage. Third, cancer cells themselves could increase the production of inflammatory cytokines, such as IL-6 and IL-8, which in turn induce the production of CRP [23]. In fact, recent reports have shown that IL-6 is associated with liver cancer progression and metastasis [24-26].

It has also been reported that serum albumin participates in the systemic inflammatory response and that a decline in its serum level is a poor prognostic factor for the long term survival in patients with various types of cancers [19, 27].

Based on these reports, the GPS, which incorporates the CRP and serum albumin levels, may reflect both the presence of the systemic inflammatory response (CRP), and the progressive nutritional decline (albumin) in patients with cancers, resulting in a poor survival outcome [27].

Consistent with Oshiro's report [13], the current study has demonstrated that an elevated GPS is independently associated with a poor prognosis in patients with BTC. In addition to Oshiro's report, we demonstrated that the GPS is an independent marker of the prognosis in patients with BTC undergoing palliative chemotherapy or BSC. These results suggest that the predictive accuracy of the GPS in patients with BTC is highly stable and independent of the treatment modality.

In the current study, a multivariate analysis revealed that the GPS was independently associated with the overall survival; however, an elevated NLR was not found to be associated with overall survival. Moreover, the

AUC analysis showed the GPS to be superior to the NLR in terms of prognostic ability. These results are inconsistent with previous studies of Gomez and Dumitrascu [12, 28]. This may be partly because, unlike their studies, the patients in our study were treated non- surgically, and the patients in our study had heterogeneity in terms of the primary tumor site. However, in a Glasgow Inflammation Outcome study, Proctor et al. demonstrated that prognostic scores based on the CRP (modified GPS) were superior to other inflammation-based prognostic scores in terms of differentiating good from poor prognostic groups in a variety of cancer types, including hepatobiliary cancer [29]. More recently, Wu et al. have also demonstrated that the GPS was superior to the NLR with respect to its prognostic value in patients with gallbladder cancer undergoing surgical resection [30].

Previous studies have reported that the surgical margins, lymph node metastasis and adjuvant chemotherapy were independent prognostic factors in patients with BTC undergoing surgical resection [31-33]. Other studies have identified the performance status, the primary tumor site, alkaline phosphatase and the presence of the metastasis as independent prognostic

factors in patients with advanced BTC not amenable to surgical resection [17, 18, 34]. Suzuki et al. demonstrated that the CRP and albumin levels were independent prognostic factors in patients with advanced BTC, in addition to the performance status [18]. This result is consistent with that of our study. We speculate that in advanced BTC patients, elevated CRP and low albumin levels could be associated with metabolic disturbance, weight loss and cachexia, thus leading to a poor outcome.

A reliable predictor of survival should be simple and easy to apply for prognostic prediction before treatment is initiated. In this regard, the GPS can be a useful tool for the prognostication and stratification of patients with BTC, because it is based on only two standard laboratory parameters, the CRP and albumin level, which are generally monitored during routine blood tests, and are available without additional imaging techniques or histological examinations before commencing treatment [35].

The current study has some potential limitations. First, it was a retrospective, small size and single-center study. Second, in our study, the diagnosis of BTC was not confirmed pathologically in 24 (46.2%) patients. A large-scale prospective validation study is therefore needed to confirm the

results of the current study.

In conclusion, we have demonstrated that the GPS, an inflammation-based prognostic score, is an independent marker of the prognosis in patients with BTC undergoing palliative chemotherapy or BSC and is superior to the mGPS and NLR in terms of prognostic ability.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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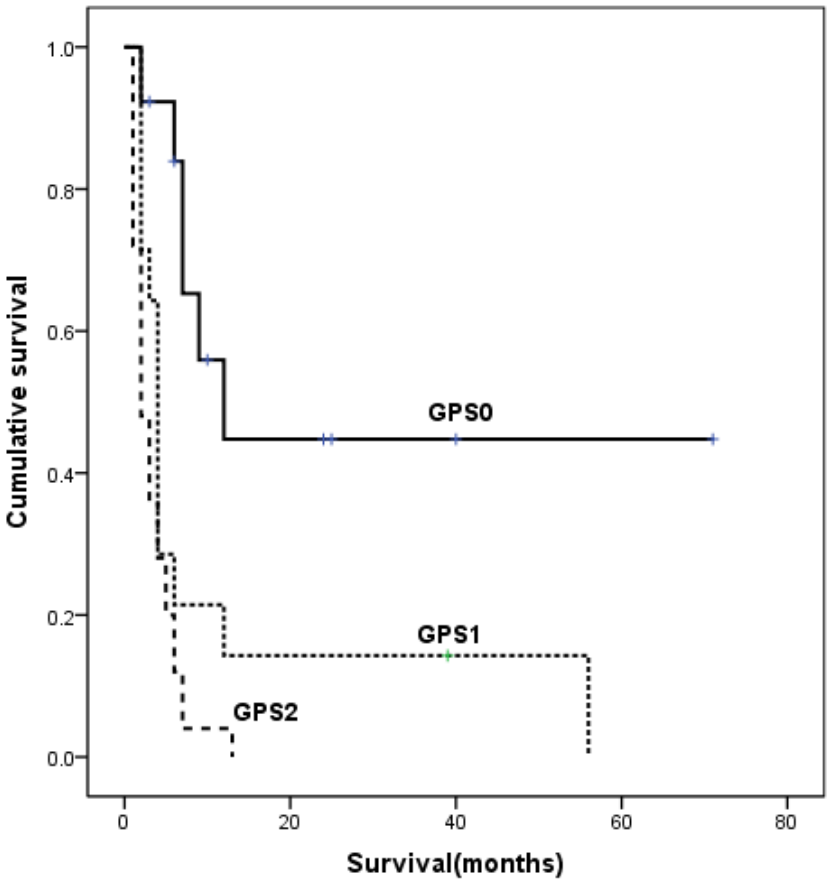
Figure Captions

Fig.1 A comparison of the cumulative survival according to the GPS.

Fig.2 A comparison of the cumulative survival according to the mGPS.

Fig.3 A comparison of the cumulative survival according to the NLR.

Fig.4 A comparison of the areas under the receiver operating curves for outcome prediction between the three scores.


Cumulative survival
1.0
0.8
0.6
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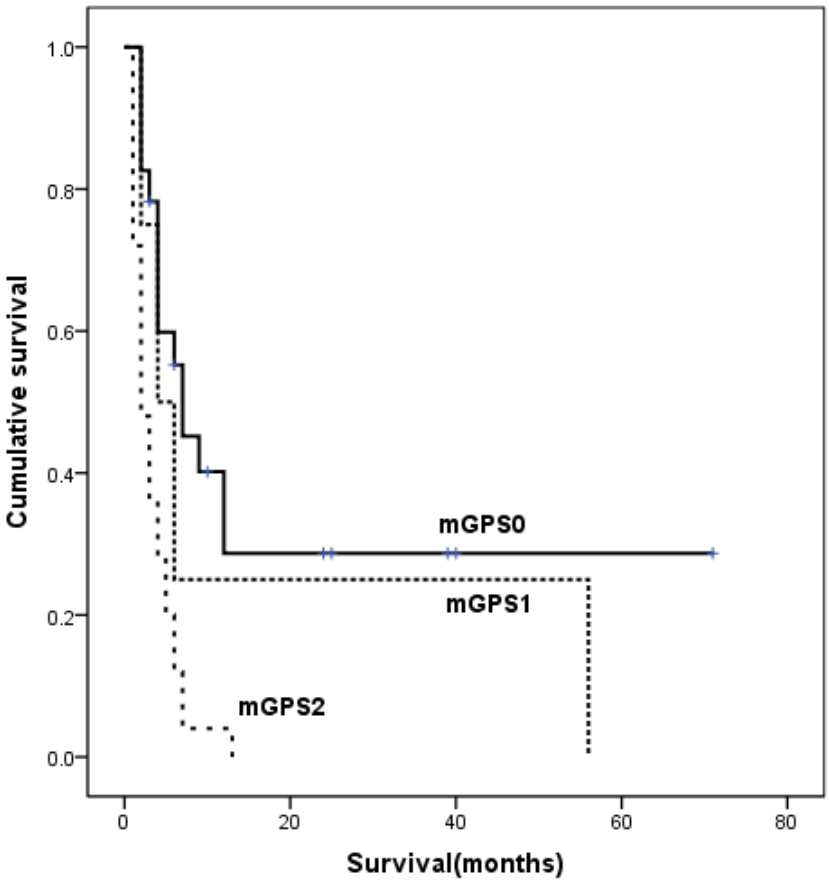
0.2
0.0
GPS0
GPS1
GPS2
0
20
40
60
80
Survival(months)

Figure 1


1.0
0.8
0.6
0.4
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Cumulative survival
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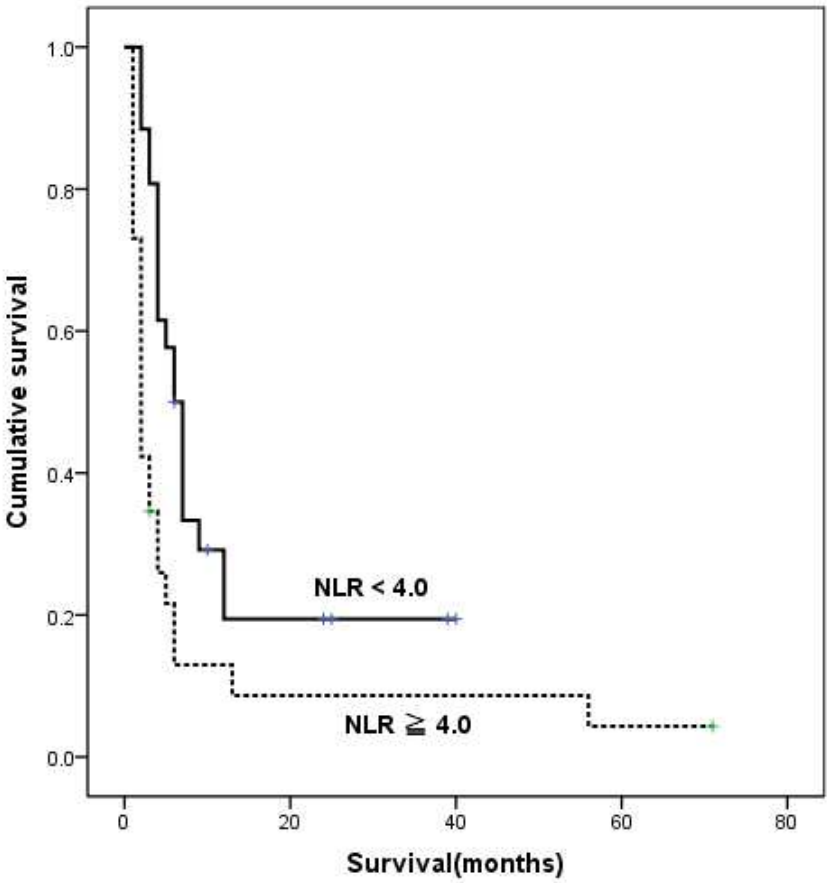
mGPS1
mGPS2
0
20
40
60
80
Survival(months)

Figure 2


Cumulative survival
1.0
0.8
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NLR < 4.0
NLR ≧ 4.0
0
20
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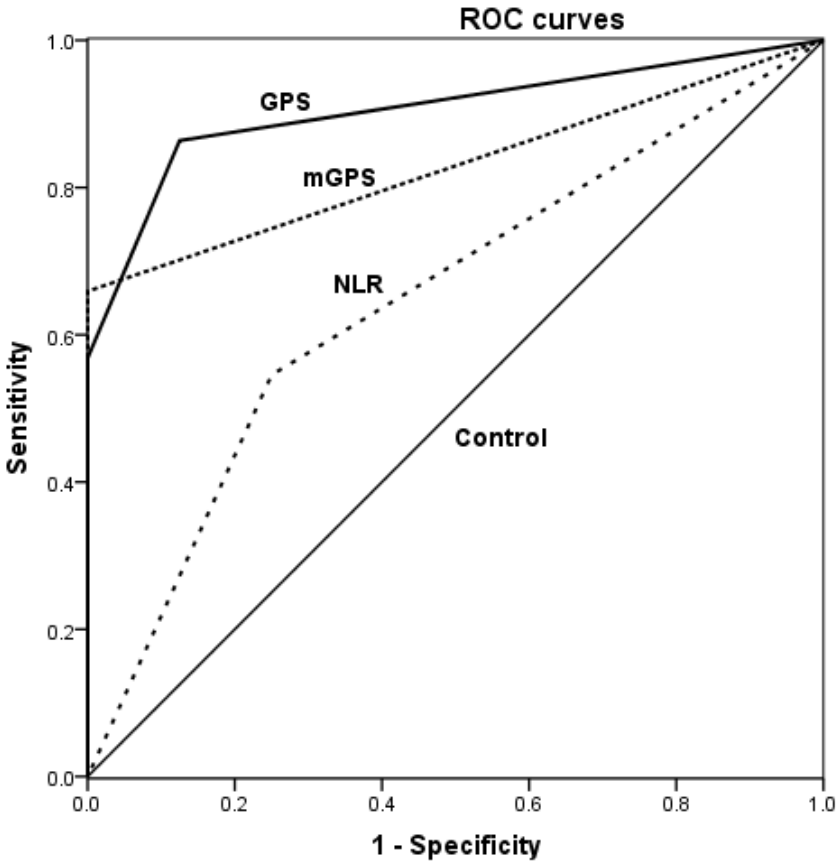
60
80
Survival(months)

Figure 3


ROC curves
GPS
mGPS
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1 - Specificity
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1.0

Figure 4

Table 1. Baseline characteristics of the patients

Variable	n=52
Age (years)	79(52-96)
Sex (male/female)	31/21
Primary tumor site (iCCA/eCCA/GBCA)	16/19/17
Metastasis (absent/present)	21/31
Biliary drainage (no/yes)	15/37
WBC ($\times 10^9$/l)	6.4(2.8-21.5)
Neutrophils ($\times 10^9$/l)	4.75(1.2-20.6)
Lymphocytes ($\times 10^{9/}$l)	1.2(0.2-4.9)
Hb (g/dl)	12.1(7.5-15.9)
Plt ($\times 10^4$/mm3)	23.8(9.4-24.0)
T-Bil (mg/dl)	2.45(0.4-28.8)
Alb (g/l)	3.1(1.6-4.8)
ALP(IU/l)	994(186-10435)
CRP (mg/dl)	1.35(0.1-25)
CEA (ng/ml)	5.75(1.6-2364)
CA19-9 (U/ml)	241.5(3-45557)
GPS (0/1/2)	13/14/25
mGPS(0/1/2)	23/4/25
NLR	3.955(0.35-43.5)
Treatment modality (chemotherapy or radiation/BSC)	21/31

Abbreviations: eCCA = extrahepatic cholangiocarcinoma; iCCA = intrahepatic cholangiocarcinoma;
GBCA = gallbladder cancer; WBC = white blood cell count; Hb = hemoglobin level;
Plt = platelet count; T-Bil = total bilirubin; Alb = albumin; ALP = alkaline phosphatase;
CRP = C-reactive protein; CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9;
GPS = Glasgow Prognostic Score; mGPS = modified GPS; NLR = neutrophil to lymphocyte ratio; BSC = best supportive care

Table 2. Clinicopathological characteristics of patients grouped according to the GPS score

Variable	GPS 0 (n=12)	GPS 1 (n=21)	GPS 2 (n=19)	P-value
Age (years)	72(52-89)	79(58-96)	80(58-94)	0.285
Sex (male/female)	9/4	10/4	12/13	0.17
Primary tumor site (iCCA/eCCA/GBCA)	4/5/4	3/5/6	9/9/7	0.868
Metastasis (absent/present)	5/8	5/9	11/14	0.695
Biliary drainage (no/yes)	7/6	3/11	6/19	0.275
WBC ($\times 10^9$/l)	5.5(4-7.7)	5.45(2.8-8.3)	9.4(4.6-21.5)	0.113
Neutrophils ($\times 10^9$/l)	3.6(1.7-5.7)	3..65(1.2-6.5)	6.9(2.9-20.6)	<0.0001
Lymphocytes ($\times 10^9$/l)	1.1(0.9-2)	1.25(0.6-3.4)	1.1(0.2-4.9)	0.915
Hb (g/dl)	13.3(11.1-15.4)	12.1(7.7-15.9)	11.2(7.5-14)	0.024
Plt ($\times 10^4$/mm3)	25.5(11.4-24.0)	22.35(10.8-34.5)	23.9(9.4-55.1)	0.631
T-Bil (mg/dl)	0.9(0.4-25.1)	2.45(0.4-14.2)	4.4(0.5-28.8)	0.313
Alb (g/l)	3.8(3.5-4.1)	3.35(2.3-4.8)	2.9(1.6-3.4)	<0.0001
ALP(IU/l)	709(219-2369)	881(186-3366)	1139(223-10435)	0.16
CRP (mg/dl)	0.6(0.1-0.9)	0.75(0.1-4.0)	5.0(1.1-25)	<0.0001
CEA (ng/ml)	6.05(1.8-427.8)	5.1(2.2-1559.6)	6.8(1.6-2363.8)	0.881
CA19-9 (U/ml)	69(3-45557)	150(1-25966)	272(1-12843)	0.291
Treatment modality (chemotherapy or radiation/BSC)	7/6	7/7	7/18	0.117

Abbreviations: eCCA = extrahepatic cholangiocarcinoma; iCCA = intrahepatic cholangiocarcinoma;
GBCA = gallbladder cancer; WBC = white blood cell count; Hb = hemoglobin level;
Plt = platelet count; T-Bil = total bilirubin; Alb = albumin; ALP = alkaline phosphata
CRP = C-reactive protein; CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9;
GPS = Glasgow Prognostic Score; mGPS = modified GPS; NLR = neutrophil to lymphocyte ratio; BSC = best supportive c

Table 3. Comparison of the areas under the curve among the three scores.

	AUC	95% CI	P-value
GPS	0.905	0.817-0.993	<0.0001
mGPS	0.83	0.718-0.942	0.003
NLR	0.648	0.448-0.848	0.187

Abbreviations: GPS = Glasgow Prognostic Score; mGPS = modified GPS; NLR = neutrophil to lymphocyte ratio;

Table 4. Prognostic factors for the overall survival of patients with BTC based on the univariate and multivariate analyses

	Univariate analysis	Multivariate analysis	
Variable	P-value	Hazard ratio (95% CI)	P-value
Age (years)	0.037		
Sex (male/female)	0.01	0.299(0.141-0.637)	0.002
Primary tumor site (iCCA/eCCA/GBCA)	0.558		
Metastasis (absent/present)	0.253		
Biliary drainage (no/yes)	0.382		
WBC ($\times 10^9$/l)	0.055		
Neutrophils ($\times 10^9$/l)	0.045		
Lymphocytes ($\times 10^9$/l)	0.569		
Hb (g/dl)	0.072		
Plt ($\times 10^4$/mm3)	0.224		
T-Bil (mg/dl)	0.519		
Alb (g/l)	0.001		
ALP(IU/l)	0.71		
CRP (mg/dl)	0.019		
CEA (ng/ml)	0.101		
CA19-9 (U/ml)	0.062		
GPS	<0.0001	2.686(1.685-4.256)	<0.0001
mGPS	<0.0001		
NLR	0.01		
Treatment modality (chemotherapy or radiation/BSC)	0.112		

Abbreviations: eCCA = extrahepatic cholangiocarcinoma; iCCA = intrahepatic cholangiocarcinoma;
GBCA = gallbladder cancer; WBC = white blood cell count; Hb = hemoglobin level;
Plt = platelet count; T-Bil = total bilirubin; Alb = albumin; ALP = alkaline phosphatase;
CRP = C-reactive protein; CEA = carcinoembryonic antigen; CA19-9 = carbohydrate antigen 19-9;
GPS = Glasgow Prognostic Score; mGPS = modified GPS; NLR = neutrophil to lymphocyte ratio; BSC = best supportive care