



Value of bile soluble B7–H3 for the diagnosis of malignant biliary strictures: Results of a retrospective study

Yang Liu^{a,1}, Chao Cheng^{a,1}, Lan Bai^b, Feng Yao^c, Shenchao Shi^d, Yu Zhang^{e,*}

^a Department of Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430022, China

^b Department of Oncology, The Sixth Hospital of Wuhan, Affiliated Hospital of Jiangnan University, Wuhan, 430014, China

^c Hepato Pancreat Obiliary & Spteen Surgery Dept Wuhan PuAi Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430000, China

^d Department of General Surgery, The Third People's Hospital of Hubei Province, Wuhan, 430033, China

^e Department of Hepatobiliary Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430022, China

ARTICLE INFO

Keywords:

B7–H3
Malignant biliary strictures
ERCP
Overall survival rate
Prognosis

ABSTRACT

Aims: Increasing evidence has demonstrated that serum soluble B7–H3 (sB7-H3) is a useful tumor marker for cancer diagnosis and prognostic evaluations. Whether sB7-H3 expressed in the bile is related to the progression of malignant biliary strictures must be clarified.

Methods: Bile sB7-H3 was obtained via endoscopic retrograde cholangiopancreatography (ERCP) from 323 patients suspected to have malignant biliary strictures and was detected using a B7–H3 ELISA kit. Diagnostic value was compared among bile sB7-H3, CA19-9, CA12-5, and CEA and ERCP-based cytological/tissue examination. Additionally, the correlations between the bile sB7-H3 concentration and the clinical characteristics of malignant biliary strictures were studied.

Results: The bile sB7-H3 levels of patients with malignant biliary strictures were significantly higher than those in patients with benign biliary strictures ($P < 0.001$). The AUC values of the receiver operating characteristic (ROC) curves for CA19-9, CA12-5 and CEA were 0.764, 0.475 and 0.399, respectively, which were significantly lower than that of sB7-H3 (0.878); the sensitivities of ERCP-based cytological and tissue examinations were 55.7% and 66.4%, respectively, which were far lower than that of bile sB7-H3 (81.2%). A high level of sB7-H3 in patients with malignant biliary strictures was found to be correlated with vascular invasion ($P < 0.001$), lymph node metastasis ($P < 0.001$), distant metastasis ($P < 0.001$) and tumor-node-metastasis (TNM) stage ($P = 0.01$). The overall survival rate of the patients in the high sB7-H3 group was significantly lower than that of the patients in the low sB7-H3 group ($P = 0.014$).

Conclusions: Bile sB7-H3 could serve as a valuable biomarker for patients with malignant biliary strictures and high levels of bile sB7-H3 were associated with poor clinical outcomes.

1. Introduction

Bile duct stricture is a common disease of the bile duct system that requires various treatments and has a variable prognosis. In the differential diagnosis of benign biliary strictures and malignant biliary strictures, the recognition of malignant strictures remains difficult in the clinical setting [1]. Malignant strictures are mainly found in the bile duct and can be due to gallbladder cancer, metastatic liver cancer, carcinoma of the head of the pancreas or periampullary carcinoma. Preoperative pathology or histopathology is the key to diagnosis. However, due to limited access to the biliary tract, acquired samples are

often insufficient, affecting the sensitivity and accuracy of diagnosis. Approximately 7%–10% of patients with suspected malignancy were found to exhibit benign postoperative pathology [2,3]. The differential diagnosis of benign and malignant biliary stenosis must be determined before considering surgical management.

For patients with suspected malignant biliary strictures, endoscopic retrograde cholangiopancreatography (ERCP) is the first-line examination. ERCP-based brush cytology and ERCP-guided transpapillary forceps biopsy can accurately diagnose malignant biliary strictures, but their sensitivities are not ideal, although the specificity of ERCP-based cytological/tissue examination is at or near 100% [4]. Alternative

* Corresponding author.

E-mail address: zhangyu754@126.com (Y. Zhang).

¹ Both authors contributed equally to this work and should be considered co-first authors.

sampling techniques are required to improve the sensitivity of diagnosis when negative ERCP-based brush cytology and ERCP-guided transpapillary forceps biopsy results are obtained. Over the past decade, endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) has been considered effective in improving the diagnostic accuracy in patients with malignant biliary strictures patients who are negative for malignancy by ERCP-based cytological/tissue examination [5,6]. However, EUS-FNA has some inherent disadvantages, including its time-consuming nature and the risks of bile leakage and tumor seeding [7,8]. Therefore, the identification of a sensitive, multidisciplinary approach based on the advantages and disadvantages of each modality is required to optimize the accuracy of the preliminary diagnosis of suspected malignant biliary strictures.

B7–H3 represents one of the B7 families. As a transmembrane protein, B7–H3 plays an important role in the T cell-mediated immune inflammatory response [9], and an increasing number of studies have found high B7–H3 expression in tumor cells, which is closely related to adverse clinical outcomes [10,11]. Soluble B7–H3 (sB7-H3) is one of the main types of B7–H3, in addition to member B7–H3(mB7-H3), and is primarily released by activated B cells, T cells, monocytes and B7–H3-positive tumor cells [12]. Its presence can be detected in peripheral blood from patients with cancer, including non-small cell type lung cancer [13], liver cancer [14] and clear cell renal cell carcinoma [15]. The expression level of serum sB7-H3 in tumor patients has been found to be significantly higher than that in healthy volunteers, and it seems to be closely related to malignant progression and prognosis [16], suggesting that sB7-H3 is a useful tumor marker for cancer diagnosis and prognosis. In the tumor microenvironment, whether tumor cells that cause biliary strictures can release sB7-H3 into the bile, whether the level of sB7-H3 in the bile in the context of malignant biliary strictures is higher than that in benign biliary strictures, and whether the expression of sB7-H3 in the bile is associated with the malignant progression and prognosis of bile duct cancer remain unclear.

In the current study, bile was collected from patients with suspected malignant biliary strictures while undergoing ERCP examination, and the diagnostic accuracy was compared between sB7-H3 and ERCP-based cytological/tissue examination. Additionally, we assessed correlations between sB7-H3 levels in bile and the clinical parameters of malignant biliary strictures.

2. Materials and methods

Consecutive patients who were diagnosed with suspected malignant biliary strictures and were treated from May 2008 to May 2014 in the Union Hospital of Huazhong University of Science and Technology were enrolled prospectively in this study. The patients included into this study underwent routine diagnostic procedures including abdominal ultrasound, abdominal CT scans and biliary tract MRI, but malignant biliary strictures could not be excluded. Then, endoscopic transpapillary brush cytology or forceps biopsy was performed, and bile was collected during ERCP procedures. Patients with negative cytology/histology after initial brush cytology or forceps biopsy subsequently underwent EUS-FNA.

Patients with positive cytology/histology underwent radical tumor resection or palliative surgery, and tumor specimens were confirmed by pathological examination. Patients diagnosed with gallbladder carcinoma, bile duct carcinoma, pancreatic cancers or periampullary adenocarcinoma were classified according to tumor-node-metastasis (TNM) classification (UICC, 2010). The study was approved by the Ethics Committee of Huazhong University of Science and Technology for Clinical Investigation. All of the patients were followed up for 3 years and detailed records of their clinical data were maintained.

3. Inclusion and exclusion criteria

The inclusion criteria were (1) a stricture of the extrahepatic bile duct revealed by CT or MRCP as a thickened bile duct wall or a pancreatobiliary mass; (2) obstructive jaundice and/or cholangitis without infection of the biliary tract; and (3) age older than 18 years old and willingness to sign the informed consent form. The exclusion criteria were (1) coagulopathy; (2) metastatic cancer; (3) ERCP contraindications; (4) intrahepatic biliary strictures; and (5) altered gastrointestinal anatomy due to previous surgery or duodenal obstruction preventing ERCP execution.

4. Diagnostic criteria

The diagnosis of malignant biliary strictures was established when (1) endoscopic transpapillary brush cytology, forceps biopsy or EUS-FNA was positive; and (2) confirmation was received via surgical pathology. Non-surgical patients considered negative for malignant biliary strictures underwent follow-up imaging studies for at least 6 months, and if any deterioration of a patient's general health was identified, a second endoscopic transpapillary forceps biopsy procedure was performed. Diagnostic accuracy was defined as the ratio of the total true-positive and true-negative results divided by the sum of the specimens. After ERCP-based brush cytology, ERCP-guided transpapillary forceps biopsy or EUS-FNA was performed, all of the patients received a follow-up examination with laboratory and radiologic tests within at least 1 day.

5. Bile acquisition and storage

During ERCP, after common bile duct cannulation and before contrast agent injection, the catheter was successfully passed through the obstructing lesion into the proximal bile duct. Then, 1–3 mL of bile were collected through the sphincterotome into a sterile syringe. Bile samples were centrifuged at 1000 g for 15 min at 4 °C, and then divided into 5 tubes and stored at –70 °C. Every three months, the sB7-H3 concentration in the bile was detected and recorded.

6. Detection of sB7-H3

The level of sB7-H3 in bile was detected using the sB7-H3 ELISA kit (R&D Systems, MIN, USA) in accordance with the operating instructions for processing. Briefly, all bile samples were added into microplate strips and incubated for 2 h, and then each well was aspirated and washed four times. Next, 200 µL of B7–H3 conjugate were transferred to each well and incubated for another 2 h at room temperature; then, they were removed and washed. Subsequently, 200 µL of substrate solution were added to each well and incubated for 30 min in the dark. Finally, 50 µL of Stop Solution was added to each well, and each sample's absorbance was detected at 450/540 nm.

7. Statistical analysis

Differences between the benign biliary strictures and malignant biliary strictures groups were evaluated by the chi-square test, Fisher's exact test or the Mann–Whitney *U* test. The diagnostic value of sB7-H3 in differentiating between benign biliary strictures and malignant biliary strictures was assessed by receiver operating characteristic (ROC) curves. The Kaplan–Meier method was used to assess the survival curves, and the log-rank test was performed to detect differences in survival rates. Factors related to prognosis in malignant biliary strictures were analyzed using univariate and multivariate analyses (Cox regression models), and covariates with *P* values less than 0.05 were included in the multivariate analysis. *P* values < 0.05 were considered statistically significant. SPSS software version 20.0 (SPSS, Chicago, IL, USA) was used for the statistical analyses.

Table 1
Patients' clinical characteristics.

	BBS	MBS	P value
Number	174	149	
Age, years	53.39 ± 11.44	54.95 ± 9.77	NS
Gender			NS
Male,n(%)	90(51.7%)	77(51.7%)	
Female,n(%)	84(48.3%)	72(48.3%)	
TBIL(μmol/L)	121.30 ± 61.56	137.45 ± 63.57	0.029
ALP(U/L)	177.33 ± 68.36	317.11 ± 78.96	0.001
ALT(U/L)	107.33 ± 26.11	113.43 ± 25.09	0.034
AST(U/L)	90.44 ± 23.92	101.18 ± 22.45	0.026
CA19-9(U/mL)	102.70 ± 40.96	147.87 ± 48.21	< 0.001
CA12-5 (U/mL)	84.59 ± 46.79	92.29 ± 43.93	< 0.001
CEA (ng/ml)	13.74 ± 7.15	11.51 ± 7.01	0.005
Stricture location			NS
Hilar	86(49.4%)	64(43.0%)	
Middle	42(24.1%)	47(31.5%)	
Distal	46(26.4%)	38(25.5%)	

All enrolled patients were divided into two groups according to pathological examination and the clinical characteristics were compared between the two groups. The levels of TBIL, ALP, ALT, AST CA19-9, CA12-5 and CEA showed significant differences between the two groups. BBS: benign biliary strictures; MBS: malignant biliary strictures.

8. Results

Detection of sB7-H3 expression in bile from patients with bile duct strictures.

A total of 323 patients with suspected malignant biliary strictures were enrolled in the study. According to ERCP-based brush cytology, ERCP-guided transpapillary forceps biopsy, EUS-FNA, postoperative specimen pathological reports and the results of follow-up imaging, all of the enrolled patients were given an exact diagnosis and were divided into two groups: benign biliary strictures (n = 174) and malignant biliary strictures (n = 149). The clinical characteristics of the two groups are shown in Table 1. The data regarding CA19-9, CA12-5, CEA and other blood biochemical examination results were obtained from the clinical laboratory before the patients received any treatment. Serum CA19-9 levels were the highest in the malignant biliary strictures group (147.87 ± 48.21 U/mL), followed by CA12-5 levels (92.29 ± 43.93 U/mL) and CEA levels (11.51 ± 7.01 ng/mL), compared to those in the benign biliary strictures group. The other biochemical indices of ALT, AST, ALP and total bilirubin in the malignant biliary strictures group also showed significant differences from those in the benign biliary strictures group. According to the narrowest points of bile duct strictures, all of the patients were divided into 4 groups: hilar, proximal, middle and distal; the numbers of benign biliary strictures in the three groups were 86, 42 and 46, respectively, while in the malignant biliary strictures group, the corresponding numbers were 64, 47 and 38, respectively. No significant difference was observed between the benign biliary strictures and malignant biliary strictures groups regarding the narrowest sites of stricture.

The sB7-H3 data of the patients were extracted from the database and analyzed. As shown in Fig. 1, the sB7-H3 levels in the bile of patients with malignant biliary strictures (3.385 ± 0.097 ng/mL, with a range of 0.682–6.431 ng/mL) were significantly higher than those in the patients with benign biliary strictures (1.619 ± 0.059 ng/mL, with a range of 0.086–3.828 ng/mL, P < 0.001).

9. The diagnostic value of bile sB7-H3 in malignant biliary strictures

To estimate the diagnostic and differential diagnostic value of sB7-H3 in the bile for benign and malignant biliary strictures, ROC analyses were performed. As shown in Fig. 2, the area under the ROC curve of bile sB7-H3 was 0.878, and the 95% confidence interval (CI) was

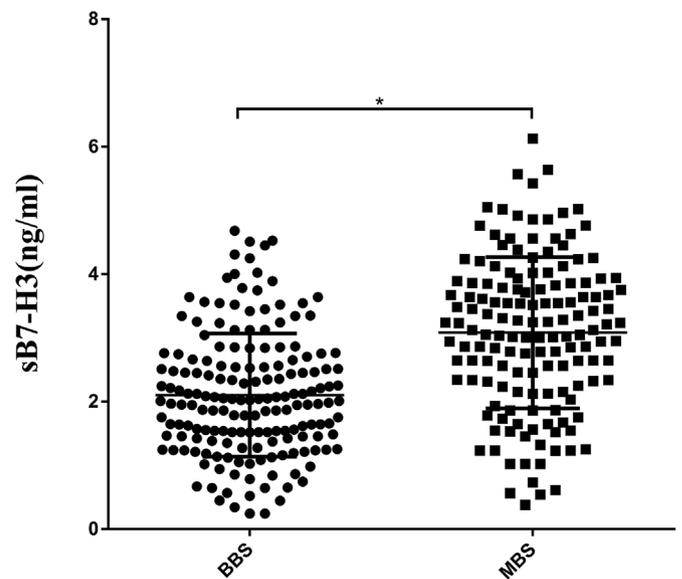


Fig. 1. Detection of sB7-H3 levels in bile samples. The concentration of sB7-H3 in bile from malignant biliary strictures patients was significantly higher than that from benign biliary strictures patients (*P < 0.001). MBS: malignant biliary strictures; BBS: benign biliary strictures.

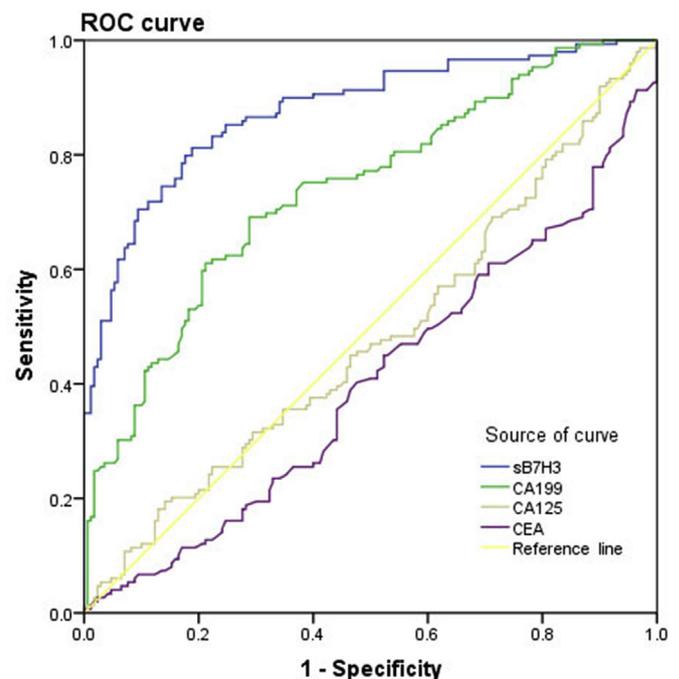


Fig. 2. Comparison of the diagnostic values of sB7-H3, CA19-9, CA12-5 and CEA in bile from malignant biliary strictures. The AUC values of bile sB7-H3, CA19-9, CA12-5 and CEA were 0.878, 0.764, 0.475 and 0.399, respectively.

0.840–0.916. The highest efficacy was detected when 2.17 ng/mL was adopted as the cut-off, with a sensitivity of 81.2%, and a specificity of 81.6%. The negative predictive value (NPV) was 85.5% and the positive predictive value (PPV) was 79.1% according to EUS-FNA and postoperative pathological results.

Furthermore, compared to bile sB7-H3 levels, we found that the areas under the ROC curves for the conventional tumor markers CA19-9, CA12-5 and CEA were 0.764, 0.475 and 0.399, respectively, which were significantly lower than that for bile sB7-H3. Additionally, their overall sensitivities and specificities for the diagnosis of malignant biliary stenosis were significantly lower than those for bile sB7-H3

Table 2
Diagnostic performance of sB7-H3, CA199, CA125 and CEA for malignant biliary strictures.

Group	Cutoff value	Sensitivity(%)	Specificity(%)	AUC	P Value*	95% CI
sB7-H3	2.17 ng/mL	81.2	81.6	0.878		0.840–0.916
CA19-9	125.04U/mL	68.4	74.7	0.764	< 0.001	0.713–0.815
CA12-5	104.58U/mL	31.5	70.6	0.475	< 0.001	0.412–0.539
CEA	14.75 ng/mL	25.5	62.6	0.399	< 0.001	0.337–0.461

The overall sensitivities and specificities for the diagnosis of malignant biliary stenosis were compared among sB7-H3, CA19-9, CA12-5 and CEA, among which sB7-H3 showed the best diagnostic performance. *P < 0.05 for comparisons of AUC values between sB7-H3 and CA19-9, between sB7-H3 and CA12-5, and between sB7-H3 and CEA.

(Table 2).

To further clarify the relationships between bile sB7-H3 levels and malignant bile duct stricture sites, we explored the diagnostic efficacy of sB7-H3 for various malignant biliary strictures at a designated cut-off level (Supplementary data 1). The diagnostic sensitivity and specificity of sB7-H3 for different narrowest sites were higher than those of CA19-9, CA12-5 and CEA, and we found that the diagnostic sensitivity of sB7-H3 for distal malignant biliary strictures was lower than that of the other 2 groups. We speculate that this finding might be due to the mixture of bile from proximal stenotic sites and pancreatic juices, which could reduce the concentration of sB7-H3.

10. Diagnostic power of bile sB7-H3 level and ERCP-based cytological/tissue examination in malignant biliary strictures

ERCP-based brush cytology and ERCP-guided transpapillary forceps biopsy are routine tests for patients with suspected malignant biliary strictures, and although they are easy to perform, their low sensitivities limit their clinical value. According to the diagnostic criteria, the sensitivity, specificity, NPV, PPV and accuracy of ERCP-based cytological/tissue examination alone or combined with sB7-H3 in differentiating malignant biliary strictures from benign biliary strictures were analyzed (Table 3). We found that the sensitivities of ERCP-based brush cytology and ERCP-guided transpapillary forceps biopsy were 55.7% and 66.4%, respectively, which were far lower than those of ERCP-based cytological/tissue examination combined with sB7-H3 in bile, with values of 83.9% and 89.3%, respectively. In addition, we studied the diagnostic performance of ERCP-based brush cytology and ERCP-guided transpapillary forceps biopsy, either alone or combined with sB7-H3, for malignant biliary strictures at different bile duct stricture sites (Supplementary data 2). We found that the sensitivity of ERCP-based cytological/tissue examination combined with sB7-H3 was far greater than that of ERCP-based brush cytology/tissue examination combined with sB7-H3 was superior to ERCP-based brush cytology and ERCP-guided transpapillary forceps biopsy alone in differentiating malignant biliary strictures from benign biliary strictures.

Table 3
Diagnostic performance of BC and TPB, either alone or combined with sB7-H3, for malignant biliary strictures.

	Sensitivity(%)	Specificity(%)	PPV(%)	NPV(%)	Accuracy(%)
BC	55.7	100	100	72.5	79.6
TPB	66.4	100	100	77.7	84.5
sB7 + BC	83.9	100	100	87.9	92.6
sB7 + TPB	89.3	100	100	91.6	95.0

The sensitivity, specificity, NPV, PPV and accuracy of ERCP-based cytological/tissue examination alone or combined with sB7-H3, in differentiating malignant biliary strictures from benign biliary strictures were analyzed. The diagnosis efficiency of sB7-H3 combined with BC or TPB was higher than that for BC or TPB alone in malignant biliary strictures. BC: brush cytology; TPB: transpapillary forceps biopsy; sB7: sB7-H3; NPV: negative predictive value; PPV: positive predictive value.

11. Correlations between bile sB7-H3 concentrations and the clinical characteristics of malignant biliary strictures

Based on the ELISA results of sB7-H3, we used 2.17 ng/mL as the cut-off value for sB7-H3 and divided the patients with malignant biliary strictures into groups with high (n = 124) and low (n = 25) sB7-H3 levels. The correlations between bile sB7-H3 levels and various clinicopathologic features and tumor-related factors were evaluated. Patients diagnosed with gallbladder carcinoma (n = 13), bile duct carcinoma (n = 98), pancreatic cancers (n = 22) or periampullary adenocarcinoma (n = 16) were classified according to the tumor-node-metastasis (TNM) classification (UICC, 2010). As shown in Table 4, a high level of bile sB7-H3 in patients with malignant biliary strictures was correlated with vascular invasion (P < 0.001), lymph node metastasis (P < 0.001), distant metastasis (P < 0.001), and TNM stage (P = 0.01). However, no statistically significant correlations were found of sB7-H3 level with tumor size, age or sex, suggesting that sB7-H3 levels could be closely related to invasion and metastasis in patients with malignant biliary strictures.

Table 4
Clinicopathological characteristics of malignant biliary strictures.

Characteristics	Number	sB7-H3 level		P value
		High, n(%)	Low, n(%)	
Age, years				NS
< 60	71	60(84.5%)	11(15.5%)	
≥ 60	78	64(82.1%)	14(17.9%)	
Gender				NS
Male	73	63(86.3%)	10(13.7%)	
Female	76	61(80.3%)	15(19.7%)	
Stricture location				NS
Hilar	64	49(76.6%)	15(23.5%)	
Middle	47	41(87.2%)	6(12.8%)	
Distal	38	34(89.5%)	4(10.5%)	
Tumor size				NS
< 3 cm	59	45(76.3%)	14(23.7%)	
≥ 3 cm	90	79(87.8%)	11(12.2%)	
Vascular invasion				< 0.001
Negative	38	21(55.3%)	17(44.7%)	
Positive	101	93(92.1%)	8(7.9%)	
Lymph node metastasis				< 0.001
Without	115	100(86.9%)	15(13.1%)	
With	34	24(70.6%)	10(29.4%)	
Distant metastasis				0.025
No	115	100(71.2%)	15(28.8%)	
Yes	34	24(89.7%)	10(10.3%)	
TNM stage				0.01
I	22	13(59.1%)	9(40.9%)	
II	42	34(80.9%)	8(19.1%)	
III	46	40(86.9%)	6(13.1%)	
IV	38	36(94.7%)	2(5.3%)	

The correlations between bile sB7-H3 levels and various clinicopathologic features and tumor-related factors were investigated. A high level of bile sB7-H3 in patients with malignant biliary strictures was correlated with vascular invasion, lymph node metastasis, distant metastasis, and TNM stage (P < 0.05).

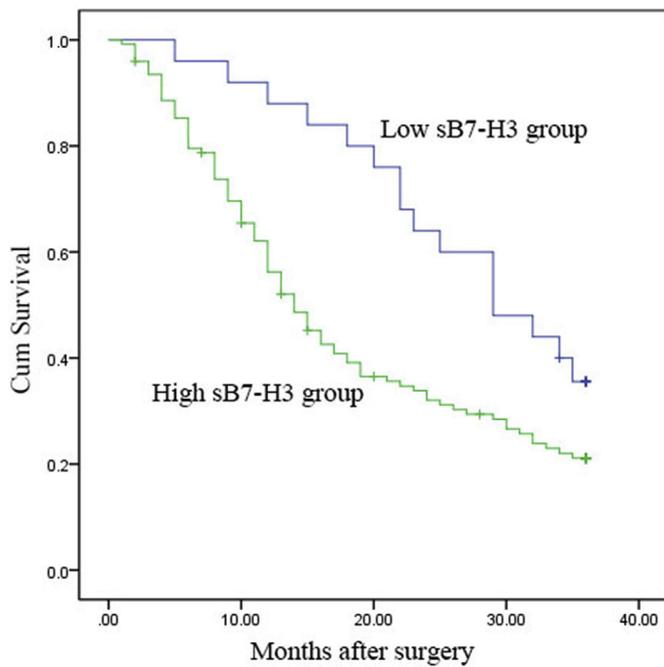


Fig. 3. OS rates for patients with malignant biliary strictures relative to bile sB7-H3 levels. All patients with malignant biliary strictures were divided into high (n = 124) and low (n = 25) sB7-H3 groups. The survival rate of the patients with malignant biliary strictures in the high sB7-H3 group was significantly lower than that of the patients in the low sB7-H3 group ($P = 0.014$).

12. Correlations between bile sB7-H3 levels and malignant biliary strictures prognosis

The median survival times of malignant biliary strictures patients with high and low sB7-H3 levels were 17.37 months and 27.24 months, respectively. The overall survival (OS) rate of the patients in the high sB7-H3 group was significantly lower than that of in the patients in the low sB7-H3 group (Fig. 3, $P = 0.014$). Additionally, as shown in Table 5, the univariate analysis revealed that the factors affecting OS were vascular invasion, lymph node metastasis, distant metastasis, TNM stage, and bile sB7-H3 level ($P = 0.024$, $P = 0.012$, $P = 0.041$, $P = 0.002$, and $P = 0.018$, respectively). The multivariate Cox regression analysis showed that the mortality risk of the patients in the high sB7-H3 group was significantly higher ($P = 0.032$), which was the same as that in the patients with vascular invasion, lymph node metastasis and a higher TNM stages ($P = 0.036$, $P = 0.022$, and $P = 0.014$, respectively).

Table 5
Univariate and multivariate analyses of OS in malignant biliary strictures patients.

Clinicopathological characteristics	Comparison/reference	Univariate analysis	P value	Multivariate analysis	P value
		HR (95% CI)		HR (95% CI)	
Age, years	≥ 60/ < 60	0.863(0.538–1.267)	NS	-	NA
Gender	Male/Female	0.796(0.463–1.085)	NS	-	NA
sB7-H3	High/Low	1.908(1.220–3.251)	0.018	1.734(1.136–2.814)	0.032
Tumor size	≥ 3/ < 3 cm	1.107(0.847–1.365)	NS	-	NA
Vascular invasion	Positive/Negative	1.854(1.174–2.974)	0.024	1.603(1.106–2.347)	0.036
Lymph node metastasis	With/Without	2.103(1.663–3.524)	0.012	1.874(1.204–2.862)	0.022
Distant metastasis	Yes/No	1.632(1.147–2.742)	0.041	1.047(0.912–1.274)	NS
TNM stage	III-IV/I-II	2.315(1.872–3.147)	0.002	2.043(1.463–2.542)	0.014

To study the factors of influence survival time with univariate and multivariate analyses. High level sB7-H3, vascular invasion, lymph node metastasis and a higher TNM stage were independent factors affecting the postsurgical survival time in patients with malignant biliary strictures. OS: overall survival; HR: hazard ratio; CI: confidence interval; NS: not significant; NA: not applicable.

13. Discussion

Bile duct stenosis is a common disease of the bile duct system. Because of the different treatment requirements and the variable prognosis, the diagnosis and differential diagnosis of benign and malignant stenosis is very important. Sensitive, convenient and rapid biological indicators of malignant biliary stenosis are lacking, and the sensitivity and accuracy of ERCP-based brush cytology and ERCP-guided transpapillary forceps biopsy are unsatisfactory [17,18]. Patients with suspected malignant biliary strictures require repeated ERCP examinations or EUS-FNA, increasing patients' medical costs and suffering, and clinical decisions can be delayed for several weeks or even months at a time [19,20]. Therefore, improving the diagnostic sensitivity and accuracy for suspected malignant biliary strictures would be conducive to clinical decisions and treatment.

In the present study, we collected bile at biliary strictures as patients with suspected malignant biliary strictures underwent ERCP, and we detected the sB7-H3 levels. After a definitive diagnosis was determined via ERCP-based brush cytology, ERCP-guided transpapillary forceps biopsy, EUS-FNA and postoperative pathologic examination, the sensitivity, specificity and accuracy of bile sB7-H3, alone or combined with ERCP-based brush cytology or ERCP-guided transpapillary forceps biopsy, were retrospectively analyzed. Bile sB7-H3 was correlated with the clinical features of malignant biliary strictures, and the value of sB7-H3 in the assessment of prognosis for malignant biliary strictures was further explored.

For the first time, the present study detected the expression of sB7-H3 in the bile after sB7-H3 was detected in serum [21] and cerebrospinal fluid [22], and the study showed that the sB7-H3 concentration in the bile in patients with malignant biliary strictures was significantly higher than in those with benign biliary strictures ($P < 0.001$). Compared to the conventional serum tumor markers CA19-9, CA12-5 and CEA, the diagnostic and differential diagnostic performance of bile sB7-H3 at the designated cut-off level was significantly greater in differentiating benign from malignant biliary stenosis and was similar at different sites of malignant biliary strictures. These results indicated that bile sB7-H3 exhibits good diagnostic performance in the differential diagnosis of benign and malignant stenosis, and sB7-H3 in bile could serve as a potential biomarker for patients with malignant biliary strictures. Bile was collected during ERCP procedures, and the sB7-H3 concentration was evaluated, which was then used alone or in combination with ERCP-based cytological/tissue examination or the diagnosis and differential diagnosis of suspected malignant biliary strictures. This method was simple and did not increase patients' pain.

ERCP-based cytological/tissue examination is the basic and primary method for patients with suspected malignant biliary strictures [23]. By observing the narrowest point and the degree of stenosis and obtaining cell and tissue samples via brush cytology or forceps biopsy, a definitive

diagnosis can be determined with high specificity in patients with suspected malignant biliary strictures. However, the diagnostic yield of ERCP-based cytological/tissue sampling for biliary stricture has been reported to be unsatisfactory due to factors such as the size and type of the stricture, stricture location, cytology preparation and interpretation, and the experience and skill of the endoscopist [24]. In this study, bile was collected during ERCP procedures, and bile sB7-H3 was detected for the diagnosis of malignant biliary strictures, overcoming the above shortcomings and enhancing the efficiency of the initial diagnosis of malignant biliary strictures. The diagnostic sensitivity and accuracy of bile sB7-H3 were significantly greater than those for ERCP-based brush cytology and ERCP-guided transpapillary forceps biopsy. Additionally, bile sB7-H3 could reduce the possibility of further EUS-FNA detection after endoscopic transpapillary brush cytology or transpapillary forceps biopsy, as well as the costs incurred by patients.

Additionally, the present study revealed correlations between bile sB7-H3 levels and the clinicopathological characteristics of patients with malignant biliary strictures. We found that the level of sB7-H3 in the bile was closely related to vascular invasion, lymph node metastasis, TNM staging and distant metastasis of the primary malignancy causing bile duct strictures, allowing for comprehensive estimation of the surgical risk and the possibility of palliative surgery. This finding also indicated that patients with high sB7-H3 levels would benefit from strong targeted chemotherapy after surgery and that patients with low sB7-H3 levels are eligible for modest postoperative chemotherapy. Additionally, our study investigated the relationship between the level of sB7-H3 in the bile and OS in patients with malignant biliary strictures, and the results showed that the 3-year OS rate in the high sB7-H3 group was significantly lower than that in the low sB7-H3 group. Moreover, the multivariable analysis confirmed that sB7-H3 in bile is an independent factor affecting the postsurgical survival time in patients with malignant biliary strictures. These results indicated that measuring sB7-H3 levels in bile could provide insight into the prognosis of patients with malignant biliary strictures.

It is worth noting that we did not evaluate B7–H3 expression in tumor tissues or the correlation between bile sB7-H3 expression levels and B7–H3 expression levels in tumor tissues in this study. Therefore, whether tumor cells can secrete sB7-H3 into the bile remains uncertain. However, the higher level of bile sB7-H3 in the patients with malignant biliary strictures compared to those with benign biliary strictures suggests that bile sB7-H3 is released from tumor cells in large amounts in patients with malignant biliary strictures. In addition, it should be noted that all of the ERCP-based cytological/tissue examinations were performed by two highly experienced endoscopists. Therefore, these results could be different in other institutions. The research findings are based on a retrospective study in a single center. Consequently, the diagnostic and prognostic value of bile sB7-H3 in patients with malignant biliary strictures requires further confirmation in prospective, multicenter studies.

14. Conclusion

The present study demonstrated that bile sB7-H3 levels could be considered an index for the differential diagnosis of benign biliary strictures and malignant biliary strictures. Moreover, sB7-H3 could be used as a biomarker for diagnosis and prognosis in patients with malignant biliary strictures.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statement

The study was approved by the Ethics Committee of Huazhong University of Science and Technology.

Acknowledgments

This study was supported by Fundamental Research Funds for the Central Universities (No. 2016YXMS250) and the Natural Science Foundation of Hubei (No. 2016CFB228), China.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.suronc.2019.01.010>.

References

- [1] S. Friman, Cholangiocarcinoma—current treatment options, *Scand. J. Surg. : SJS : Off. Organ Finnish Surg. Soc. Scand. Surg. Soc.* 100 (2011) 30–34.
- [2] A. Gerritsen, I.Q. Molenaar, T.L. Bollen, C.Y. Nio, M.G. Dijkgraaf, H.C. van Santvoort, et al., Preoperative characteristics of patients with presumed pancreatic cancer but ultimately benign disease: a multicenter series of 344 pancreatoduodenectomies, *Ann. Surg. Oncol.* 21 (2014) 3999–4006.
- [3] S.C. Abraham, R.E. Wilentz, C.J. Yeo, T.A. Sohn, J.L. Cameron, J.K. Boitnott, et al., Pancreaticoduodenectomy (Whipple resections) in patients without malignancy: are they all 'chronic pancreatitis'? *Am. J. Surg. Pathol.* 27 (2003) 110–120.
- [4] M. de Bellis, S. Sherman, E.L. Fogel, H. Cramer, J. Chappo, L. McHenry Jr. et al., Tissue sampling at ERCP in suspected malignant biliary strictures (Part 2), *Gastrointest. Endosc.* 56 (2002) 720–730.
- [5] M.J. Hewitt, M.J. McPhail, L. Possamai, A. Dhar, P. Vlavianos, K.J. Monahan, EUS-guided FNA for diagnosis of solid pancreatic neoplasms: a meta-analysis, *Gastrointest. Endosc.* 75 (2012) 319–331.
- [6] Y.N. Lee, J.H. Moon, H.K. Kim, H.J. Choi, M.H. Choi, D.C. Kim, et al., Core biopsy needle versus standard aspiration needle for endoscopic ultrasound-guided sampling of solid pancreatic masses: a randomized parallel-group study, *Endoscopy* 46 (2014) 1056–1062.
- [7] S.C. Paquin, G. Gariepy, L. Lepanto, R. Bourdages, G. Raymond, A.V. Sahai, A first report of tumor seeding because of EUS-guided FNA of a pancreatic adenocarcinoma, *Gastrointest. Endosc.* 61 (2005) 610–611.
- [8] S. Doi, I. Yasuda, T. Iwashita, T. Ibuka, H. Fukushima, H. Araki, et al., Needle tract implantation on the esophageal wall after EUS-guided FNA of metastatic mediastinal lymphadenopathy, *Gastrointest. Endosc.* 67 (2008) 988–990.
- [9] A.I. Chapoval, J.N. B7-H3, A costimulatory molecule for T cell activation and IFN- γ production, *Nat. Immunol.* 2 (2001) 269–274.
- [10] G. Chavin, Y. Sheinin, P.L. Crispin, S.A. Boorjian, T.J. Roth, L. Rangel, et al., Expression of immunosuppressive B7-H3 ligand by hormone-treated prostate cancer tumors and metastases, *Clin. Canc. Res. : Off. J. Am. Assoc. Canc. Res.* 15 (2009) 2174–2180.
- [11] E. Picarda, K.C. Ohaegbulam, X. Zang, Molecular pathways: targeting B7-H3 (CD276) for human cancer immunotherapy, *Clin. Canc. Res. : Off. J. Am. Assoc. Canc. Res.* 15 (2016) 3425–3431.
- [12] G. Zhang, J. Hou, J. Shi, G. Yu, B. Lu, X. Zhang, Soluble CD276 (B7-H3) is released from monocytes, dendritic cells and activated T cells and is detectable in normal human serum, *Immunology* 123 (2008) 538–546.
- [13] G. Zhang, Y. Xu, X. Lu, H. Huang, Y. Zhou, B. Lu, et al., Diagnosis value of serum B7-H3 expression in non-small cell lung cancer, *Lung Canc.* 66 (2009) 245–249.
- [14] W. Chen, P. Liu, Y. Wang, W. Nie, Z. Li, W. Xu, et al., Characterization of a soluble B7-H3 (sB7-H3) spliced from the intron and analysis of sB7-H3 in the sera of patients with hepatocellular carcinoma, *PLoS One* 8 (2013) e76965.
- [15] A. Masuda, K. Arai, D. Nishihara, T. Mizuno, H. Yuki, T. Kambara, et al., Clinical significance of serum soluble T cell regulatory molecules in clear cell renal cell carcinoma, *BioMed Res. Int.* 2014 (2014) 396064.
- [16] L. Zhao, C. Xie, D. Liu, T. Li, Y. Zhang, C. Wan, Early detection of hepatocellular carcinoma in patients with hepatocirrhosis by soluble B7-H3, *J. Gastrointest. Surg. : Off. J. Soc. Surg. Aliment. Tract* 21 (2017) 807–812.
- [17] A. Larghi, I. Waxman, Differentiating benign from malignant idiopathic biliary strictures: are we there yet? *Gastrointest. Endosc.* 66 (2007) 97–99.
- [18] U. Navaneethan, B. Njei, V. Lourdasamy, R. Konjeti, J.J. Vargo, M.A. Parsi, Comparative effectiveness of biliary brush cytology and intraductal biopsy for detection of malignant biliary strictures: a systematic review and meta-analysis, *Gastrointest. Endosc.* 81 (2015) 168–176.
- [19] A. Fritscher-Ravens, D.C. Broering, W.T. Knoefel, X. Rogiers, P. Swain, F. Thonke, et al., EUS-guided fine-needle aspiration of suspected hilar cholangiocarcinoma in potentially operable patients with negative brush cytology, *Am. J. Gastroenterol.* 99 (2004) 45–51.
- [20] M. Mohamadnejad, J.M. DeWitt, S. Sherman, J.K. LeBlanc, H.A. Pitt, M.G. House, et al., Role of EUS for preoperative evaluation of cholangiocarcinoma: a large single-center experience, *Gastrointest. Endosc.* 73 (2011) 71–78.
- [21] T. Arigami, Y. Uenosono, M. Hirata, S. Yanagita, S. Ishigami, S. Natsugoe, B7-H3 expression in gastric cancer: a novel molecular blood marker for detecting circulating tumor cells, *Cancer Sci.* 102 (2011) 1019–1024.
- [22] A. Baral, H.X. Ye, P.C. Jiang, Y. Yao, Y. Mao, B7-H3 and B7-H1 expression in cerebral spinal fluid and tumor tissue correlates with the malignancy grade of glioma patients, *Oncology letters* 8 (2014) 1195–1201.
- [23] J. Jaiwala, E.L. Fogel, S. Sherman, K. Gottlieb, J. Flueckiger, L.G. Bucksot, et al., Triple-tissue sampling at ERCP in malignant biliary obstruction, *Gastrointest. Endosc.* 51 (2000) 383–390.
- [24] J.H. Lee, R. Salem, H. Aslanian, M. Chacho, M. Topazian, Endoscopic ultrasound and fine-needle aspiration of unexplained bile duct strictures, *Am. J. Gastroenterol.* 99 (2004) 1069–1073.