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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Procalcitonin is a useful biomarker to predict severe acute cholangitis: a single-center prospective study

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Abstract

Background Procalcitonin is being increasingly used to diagnose and grade acute systemic bacterial infection at an early stage of disease onset. The aim of this prospective study was to evaluate the usefulness of procalcitonin for severity grading of acute cholangitis on patient admission.

Methods Patients with acute cholangitis were prospectively enrolled. The severity of acute cholangitis was graded on the basis of the 2013 Tokyo guidelines (Japanese Society of Hepato-Biliary-Pancreatic Surgery, 2013). We compared the ability of procalcitonin level on admission to predict moderate/severe (vs mild) or severe (vs mild/moderate) acute cholangitis with the abilities of white blood cell (WBC) count and C-reactive protein (CRP) level.

Results Two hundred thirteen patients were analyzed, and the severity of acute cholangitis was graded as mild, moderate, and severe in 108, 76, and 29 patients respectively. Procalcitonin level, WBC count, and CRP level all increased significantly according to the severity. In the receiver operating characteristic analyses, the area under the curve for procalcitonin for severe acute cholangitis was 0.90 [95% confidence interval (CI) 0.85–0.96] and was significantly greater than that for WBC (0.62; 95% CI 0.48–0.76) and that for CRP (0.70; 95% CI 0.60–0.80). The optimal cutoff value for procalcitonin for prediction of severe acute cholangitis was 2.2 ng/mL (sensitivity 0.97; specificity 0.73; accuracy 0.77). The areas under the curve for procalcitonin, WBC, and CRP for moderate/severe acute cholangitis were not significantly different.

Conclusions Procalcitonin predicted severe acute cholangitis better than conventional biomarkers. Severe cases for which urgent biliary drainage is indicated might be identified on admission on the basis of the cutoff values for procalcitonin suggested in this study.

Keywords Procalcitonin · Cholangitis · Choledocholithiasis · Endoscopic retrograde cholangiopancreatography

Abbreviations

AUC	Area under the curve
CRP	C-reactive protein
IQR	Interquartile range
PCT	Procalcitonin
ROC	Receiver operating characteristic
TG13	2013 Tokyo guidelines
WBC	White blood cell

Introduction

Acute cholangitis is a bacterial infection of the biliary tract which is frequently encountered in daily clinical practice, and usually develops because of biliary obstruction (e.g., choledocholithiasis, stent occlusion, biliary strictures) and

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resultant cholangiovenous reflux [1, 2]. This emergency disease is potentially lethal when accompanied by sepsis, and prompt risk stratification is mandatory to facilitate timely treatments and improve clinical outcomes. Treatment of acute cholangitis is initiated by resuscitation with intravenous fluid therapy and antibiotics, but emergent biliary drainage is required to decompress the bile duct of patients with severe acute cholangitis or those who are not amenable to conservative treatments [3–6]. In the 2013 Tokyo guidelines (TG13) for acute cholangitis and acute cholecystitis (Japanese Society of Hepato-Biliary-Pancreatic Surgery, 2013), early biliary drainage (within 12 h) is recommended for moderate cases, and urgent drainage (within 6 h) is recommend for severe cases [7]. It is therefore very important to identify predictive factors for high-risk patients who require emergent biliary drainage for acute cholangitis at an early stage of disease onset.

Procalcitonin is a 116 amino acid protein, and is a precursor of calcitonin. Under conditions without systemic bacterial or fungal infection, calcitonin is produced predominantly by C cells of the thyroid gland, and serum procalcitonin is not detectable (less than 0.05 ng/mL). In cases with systemic bacterial infection, procalcitonin is readily produced by various organs throughout the body (e.g., lung, kidney, liver, adipose cell, and muscle) immediately after disease onset (usually within 6–12 h), and serum procalcitonin level increases dramatically [8–11]. Accumulating evidence suggests that procalcitonin level well predicts the presence and severity of acute systemic infectious diseases with superior sensitivity and specificity compared with conventional inflammation markers such as white blood cell (WBC) count and C-reactive protein (CRP) level, particularly in respiratory tract infection or severe sepsis in intensive care units [12–17]. Given that acute cholangitis can provoke acute-onset systemic bacterial inflammation, we hypothesized that the level of procalcitonin might better predict the severity of acute cholangitis compared with widely used conventional biomarkers. To test this hypothesis, we conducted a prospective study to evaluate the usefulness of the level of procalcitonin as a biomarker to predict the severity of acute cholangitis on patient admission compared with WBC count and CRP level.

Methods

Study design

We conducted a single-center prospective study to evaluate the usefulness of procalcitonin level on admission to predict the severity of acute cholangitis. The primary outcome of this study was the ability of procalcitonin level to predict severe (vs mild/moderate) or moderate/severe (vs mild) cholangitis compared with WBC count and CRP level, which have been most frequently used as inflammatory biomarkers for bacterial infection in Japan [12–17]. The secondary outcomes included the ability of procalcitonin level to predict severe acute cholangitis due to choledocholithiasis or stent occlusion and for positive blood culture.

This study was approved by the Institutional Review Board of the University of Tokyo Hospital, was registered in University Hospital Medical Information Network Clinical Trials Registry (clinical trial registration number UMIN000010202), and was conducted according to the guidelines in the Helsinki Declaration. Written informed consent was obtained from all patients.

Patients

Adult patients (20 years or older) in whom acute bacterial cholangitis was diagnosed and who were hospitalized between December 2012 and September 2015 were screened for inclusion and exclusion criteria. In patients who underwent multiple hospitalizations because of acute cholangitis during the study period, the initial hospitalization was included in the analysis. Patients were excluded if they took oral antibiotics before a hospital visit due to the index episode of acute cholangitis. Patients with a percutaneous biliary catheter in situ or a history of choledochojejunostomy were excluded from the analysis because they were likelier to undergo repeated episodes of acute cholangitis and contamination by multiple-drug-resistant bacteria.

Examinations on admission

Procalcitonin level was examined on admission and blood tests were performed for the following: complete blood count (WBC, hemoglobin, and platelets), CRP, alkaline phosphatase, γ-glutamyl transpeptidase, aspartate aminotransferase, alanine aminotransferase, total bilirubin, albumin, creatinine, amylase, and lipase levels, and prothrombin time. The normal ranges for procalcitonin, WBC, and CRP in our institution were 0.49 ng/mL or less, 3300–8600/μL, and 0.30 mg/dL or less respectively. Abdominal ultrasonography, computed tomography, and/or magnetic resonance cholangiopancreatography was performed to determine the cause of acute cholangitis. Two sets of blood culture for aerobic and anaerobic bacteria were obtained, if indicated, and intravenous antibiotics were administered in all cases. In patients who underwent endoscopic or percutaneous biliary drainage, the bile aspirated through a catheter was submitted for culture of aerobic and anaerobic bacteria.

Diagnosis of acute cholangitis and the severity

Acute cholangitis was diagnosed according to TG13 for acute cholangitis and acute cholecystitis [18]. In brief, the diagnostic criteria include the presence of systemic inflammation (fever, chill, an elevated/decreased WBC count, or an elevated level of CRP), cholestasis (jaundice or an elevated level of liver enzymes), and findings of abdominal imaging (biliary dilation or evidence of potential causes of biliary obstruction). The severity was determined on the basis of TG13 for diagnosis and severity grading of acute cholangitis (Table S1), and categorized as mild (grade I), moderate (grade II), or severe (grade III) [18]. The severity in patients with prolonged leukopenia (WBC count less than 4000/μL, one of the factors for the diagnostic criteria for moderate acute cholangitis) or thrombopenia (platelet count less than 100,000/μL, one of the factors for the diagnostic criteria for severe acute cholangitis) due to chemotherapy was graded by factors other than WBC count or platelet count to avoid overestimation.

The time to admission was defined as follows: 0 days for admission within 24 h of onset of symptoms or for acute cholangitis which developed during hospitalization, 1 day for admission within 24–48 h, 2 days for admission within 48–72 h, and so forth.

Measurement of procalcitonin

Peripheral blood samples were obtained on admission, and the serum level of procalcitonin was measured with an electrochemiluminescence immunoassay system (Elecsys B.R.A.H.M.S. procalcitonin assay; Roche Diagnostics Japan, Tokyo, Japan) according to the manufacturer's instructions [19]. This is a fully automated, sensitive, quantitative procalcitonin assay system. The analytical sensitivity (i.e., limit of detection) for procalcitonin was 0.02 ng/mL, and the measuring range was 0.02–100 ng/mL. The assay time was 18 min, and results were available within 1 h.

Statistical analyses

All statistical analyses were performed with R, version 2.15.1 (R Development Core Team; http://www.r-project.org), and its *pROC* and *rms* packages. A two-sided *P* value of less than 0.05 was considered statistically significant.

The primary hypothesis testing of this study was to compare the area under the curve (AUC) for procalcitonin with that for WBC or CRP in receiver operating characteristic (ROC) curves for severe (vs mild/moderate) or moderate/severe (vs mild) cholangitis. The AUC were compared between groups by the Delong test. Taking multiple hypothesis testing into account, we adjusted the *P* value for the primary hypothesis testing by the Bonferroni correction, and the significance level was set to 0.013 (i.e., $P = 0.05/4$). The optimal cutoff values for procalcitonin in the ROC curves were determined on the basis of the Youden index. Taking the exploratory nature of secondary and exploratory analyses into account, we did not adjust the *P* values for those analyses, and the results were interpreted conservatively.

Continuous variables were expressed as medians and interquartile ranges (IQRs), and categorical variables were expressed as numbers and percentages of patients. Continuous variables were compared with the Mann–Whitney *U* test or the Kruskal–Wallis test, as appropriate, and categorical variables were compared with Fisher's exact test or the chi-square test, as appropriate. The Jonckheere–Terpstra test was used to assess the trend of each inflammatory biomarker according to the severity of acute cholangitis. The Cochran–Armitage test was used to assess the trend of severity according to the number of species identified by culture. A multivariate logistic regression model was applied to examine the relationship between procalcitonin level and severe acute cholangitis, with adjustment for the cause of acute cholangitis (choledocholithiasis, stent occlusion, or biliary stricture). The relationship between procalcitonin level and the severity of acute cholangitis might not be linear, and thus we used a restricted cubic spline function to fit the potential nonlinear relationship of procalcitonin level with four knots (5th, 25th, 75th, and 95th percentiles) [20]. The reference value for procalcitonin was set to 0.5 ng/mL, which is commonly used as a cutoff value to predict systemic infection or sepsis [21–25].

Results

Patient characteristics

Among 326 acute cholangitis patients screened, 62 patients did not give their consent for their inclusion in the study, and so 264 patients were enrolled in the prospective study. We excluded 51 patients with a percutaneous biliary catheter in situ or with a history of choledochojejunostomy, leaving a total of 213 patients as our study population (Fig. 1). Table 1 summarizes the baseline characteristics and clinical outcomes of the total study population. There were 131 men and 82 women, with a median age of 74 years. Acute cholangitis was graded as mild, moderate, and severe in 108 patients (51%), 76 patients (36%), and 29 patients (14%) respectively. Stent occlusion was the commonest cause of acute cholangitis (50%) and was associated with lower severity of acute cholangitis. Patients with

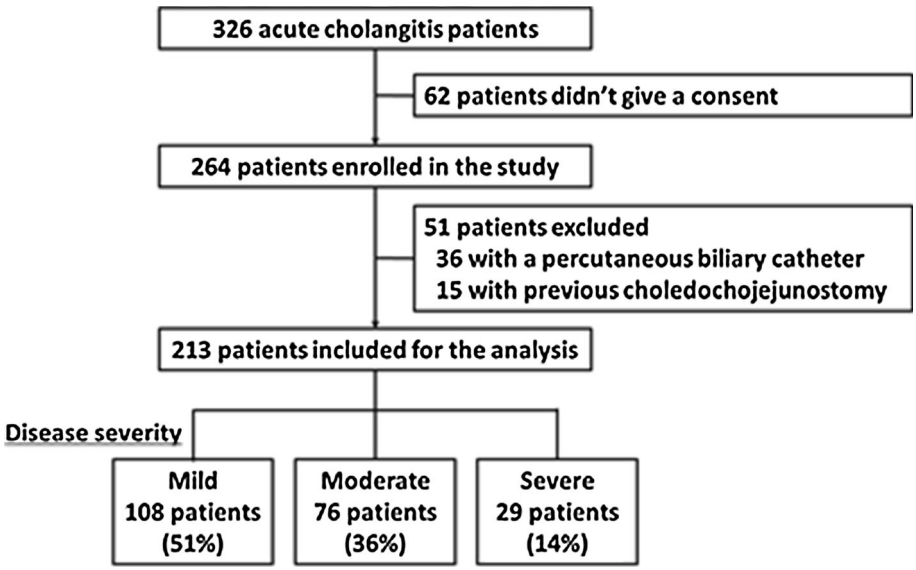

Fig. 1 Flowchart for inclusion of patients with acute cholangitis. The severity of acute cholangitis (mild, moderate, or severe) was determined on the basis of the 2013 Tokyo guidelines for diagnosis and severity grading of acute cholangitis [18]

mild acute cholangitis were younger than those with moderate or severe acute cholangitis. Compared with patients with mild or moderate acute cholangitis, patients with severe acute cholangitis were likelier to have positive blood culture results. According to the definition of severe acute cholangitis, cardiovascular dysfunction, disturbance of consciousness, and respiratory dysfunction were observed only in severe cases. Biliary drainage was performed by endoscopic procedures in all patients in whom it was indicated.

In the total study population, the following treatments were administered: chemotherapy in 64 patients (30%), nonsteroidal anti-inflammatory drugs in 23 patients (11%), acetoaminophen in 6 patients (2.8%), steroids in 6 patients (2.8%), and immunosuppressive drugs in 2 patients (0.9%; taclolimus and salazosulfapyridine each in 1 patient). The distributions of those medications were not different between the subgroups according to the severity of acute cholangitis.

Bacteria as pathogens in acute cholangitis and antibiotic administration

Blood and bile cultures were obtained in 170 patients (80%) and 189 patients (89%) respectively (Table 1). Although those cultures were performed at the discretion of attending physicians, the rate of blood and bile culture performed did not differ according to the disease severity. Table 2 summarizes the bacteria identified by blood and bile cultures. We examined the severity of acute cholangitis and procalcitonin level according to the bacteria identified by blood and bile cultures (Table 3). Positive blood culture was associated with severe acute cholangitis. Positive blood culture, an increased number of species in blood culture, positive bile culture, and positive anaerobic bacteria culture were associated with a higher level of procalcitonin. The antibiotics initially administered are summarized in Table S2. Tazobactam/piperacillin and flomoxef were the two most frequently used antibiotics in our institution.

Trend of inflammatory biomarkers (procalcitonin, WBC, and CRP) according to the severity of acute cholangitis

Figure 2 illustrates the trends of procalcitonin level, WBC count, and CRP level according to the severity of acute cholangitis. The median value of each biomarker was as follows: procalcitonin, 0.45 ng/mL (IQR 0.22–1.69 ng/mL), 1.25 ng/mL (IQR 0.41–4.18 ng/mL), and 19.51 ng/mL (IQR 4.41–53.36 ng/mL) in mild, moderate, and severe acute cholangitis respectively; WBC, 7900/μL (IQR, 6200–9900/μL), 12,300/μL (IQR 8100–14,400/μL), and 12,000/μL (IQR 5900–16,800/μL) in mild, moderate, and severe acute cholangitis respectively; CRP, 3.4 mg/dL (IQR 1.3–6.6 mg/dL), 6.1 mg/dL (IQR 3.0–11.3 mg/dL), and 7.7 mg/dL (IQR 4.8–17.1 mg/dL) in mild, moderate, and severe acute cholangitis respectively. The trend for an increase in the levels of all the inflammatory biomarkers according to the severity of acute cholangitis was statistically significant ($P < 0.0001$; Fig. 2).

Ability of procalcitonin level to predict the severity of acute cholangitis compared with WBC count or CRP level

Figure 3 illustrates ROC curves for procalcitonin, WBC, and CRP to predict severe (vs mild/moderate) or moderate/severe (vs mild) acute cholangitis. The AUC for procalcitonin for severe acute cholangitis was significantly greater than

Table 1 Characteristics and outcomes of 213 patients with acute cholangitis according to disease severity determined on the basis of the 2013 Tokyo guidelines for diagnosis and severity grading of acute cholangitis [18]

	All cases ($n = 213$)	Severity of acute cholangitis			P
		Mild ($n = 108$)	Moderate ($n = 76$)	Severe ($n = 29$)	
Characteristic					
Age (years)	74 (66–81)	69 (61–74)	80 (74–85)	79 (72–86)	<0.0001
Sex, male/female	131/82 (62%/38%)	62/46 (57%/43%)	51/25 (67%/33%)	18/11 (62%/38%)	0.41
Body temperature (°C)	38.3 (37.8–39.0)	38.5 (38.0–39.0)	38.3 (37.4–39.0)	38.1 (37.0–39.0)	0.32
Systolic blood pressure (mmHg)	116 (104–132)	118 (107–132)	121 (105–134)	104 (90–120)	0.021
Abdominal pain	71 (33%)	35 (32%)	23 (30%)	13 (45%)	0.36
Cardiovascular dysfunction[a]	9 (4.2%)	0	0	9 (31%)	–
Disturbance of consciousness[a]	10 (4.7%)	0	0	10 (34%)	–
Respiratory dysfunction[a]	11 (5.2%)	0	0	11 (38%)	–
Cause					
Stent occlusion	107 (50%)	61 (56%)	41 (54%)	5 (17%)	0.0005
Choledocolithiasis	82 (38%)	30 (28%)	30 (39%)	22 (76%)	<0.0001
Other	24 (12%)	17 (16%)	5 (6.6%)	2 (6.9%)	0.14
Use of catecholamine	9 (4.2%)	0	0	9 (31%)	–
Chemotherapy	64 (30%)	45 (42%)	16 (21%)	3 (10%)	0.0004
Steroid	6 (2.8%)	1 (0.9%)	4 (5.3%)	1 (3.4%)	0.18
Acetaminophen	6 (2.8%)	2 (1.9%)	3 (3.9%)	1 (3.4%)	0.52
NSAID	23 (11%)	8 (7.4%)	11 (14%)	4 (14%)	0.24
Immunosuppressive drug	2 (0.9%)	1 (0.9%)	1 (1.3%)	0	1.00
Laboratory test					
Procalcitonin (ng/mL)	0.86 (0.29–4.55)	0.45 (0.22–1.69)	1.25 (0.41–4.18)	19.51 (4.41–53.36)	<0.0001
WBC (/μL)	9500 (6600–12,600)	7900 (6200–9900)	12,300 (8100–14,400)	12,000 (5900–16,800)	<0.0001
CRP (mg/dL)	4.9 (1.9–9.5)	3.4 (1.3–6.6)	6.1 (3.0–11.3)	7.7 (4.8–17.1)	<0.0001
Total bilirubin (mg/dL)	2.7 (1.5–4.9)	2.3 (1.3–4.3)	2.9 (1.8–6.1)	3.9 (2.7–6.7)	0.0013
Albumin (mg/dL)	3.3 (2.9–3.6)	3.4 (3.0–3.7)	3.0 (2.6–3.4)	3.3 (3.1–3.5)	<0.0001
Creatinin (mg/dL)	0.8 (0.6–1.0)	0.7 (0.6–0.9)	0.8 (0.7–1.1)	1.3 (0.8–2.2)	<0.0001
Platelets ($\times 10^4$/μL)	17.8 (13.6–24.8)	17.2 (13.7–25.8)	20.4 (15.6–25.6)	11.5 (8.3–17.8)	<0.0001
PT-INR	1.05 (0.97–1.20)	1.04 (0.95–1.12)	1.05 (0.97–1.20)	1.30 (1.09–1.65)	<0.0001
Outcome					
Blood culture performed	170 (80%)	83 (77%)	62 (82%)	25 (86%)	0.51
Positive blood culture[b]	58 (34%)	22 (27%)	19 (31%)	17 (68%)	0.0007
Bile culture performed	189 (89%)	92 (85%)	70 (92%)	27 (93%)	0.29
Positive bile culture[b]	174 (92%)	82 (89%)	65 (93%)	27 (100%)	0.21
Time to admission (days)	0 (0–1)	0 (0–1)	0 (0–2)	0 (0–1)	0.13
Length of antibiotic therapy (days)	8 (6–12)	8 (5–11)	8 (6–12)	9 (7–15)	0.071
Length of stay (days)	13 (10–23)	12 (10–19)	14 (10–25)	17 (12–25)	0.039
In-hospital deaths	0	0	0	0	1.00
Biliary drainage					
Biliary drainage performed	203 (95%)	102 (94%)	74 (97%)	27 (93%)	0.49
Urgent[c]	132 (62%)	61 (56%)	46 (61%)	25 (86%)	0.0096
Early[c]	71 (33%)	41 (38%)	28 (37%)	2 (6.9%)	0.0024
Indication for biliary drainage[d]					
High temperature (≥39 °C)	62 (31%)	31 (30%)	22 (30%)	9 (33%)	0.95

Table 1 continued

	All cases ($n = 213$)	Severity of acute cholangitis			P
		Mild ($n = 108$)	Moderate ($n = 76$)	Severe ($n = 29$)	
Jaundice (total bilirubin ≥5 mg/dL)	50 (25%)	14 (14%)	26 (35%)	10 (37%)	0.0011
Shivering	39 (19%)	18 (18%)	14 (19%)	7 (26%)	0.62
Disturbance of consciousness	10 (4.9%)	0	0	10 (37%)	–
Other	82 (40%)	50 (49%)	25 (34%)	7 (26%)	0.033
Plastic stent[d]	69 (34%)	22 (22%)	27 (36%)	20 (74%)	<0.0001
Type[e]					
Straight tip	68 (99%)	22 (100%)	26 (96%)	20 (100%)	1.00
Pig-tailed tip	1 (1.4%)	0	1 (3.7%)	0	1.00
Diameter[e]					
7 F/8.5 F/10 F	26/36/7 (38%/52%/10%)	9/7/6 (41%/32%/27%)	9/17/1 (33%/63%/3.7%)	8/12/0 (40%/60%/0%)	0.025
Length[e]					
7 cm/10 cm/12 cm	59/6/4 (86%/8.7%/5.8%)	19/2/1 (86%/9.1%/4.5%)	22/2/3 (81%/7.4%/11%)	18/2/0 (90%/10%/0%)	0.73
Nasobiliary catheter[d]	130 (64%)	78 (76%)	45 (61%)	7 (26%)	<0.0001
Type[e]					
Straight tip	51 (39%)	32 (41%)	17 (38%)	2 (29%)	0.87
Pig-tailed tip	79 (61%)	46 (59%)	28 (62%)	5 (71%)	0.87
Diameter[e]					
5–6 F/7 F	67/63 (52%/48%)	44/34 (56%/44%)	21/24 (47%/53%)	2/5 (29%/71%)	0.28

Values are expressed as the number and percentage in *parentheses* or the median and the interquartile range in *parentheses*. The percentage indicates the proportion of patients with a specific baseline characteristic or clinical outcome among each severity group unless otherwise indicated.

CRP C-reactive protein, *NSAID* nonsteroidal anti-inflammatory drug, *PT-INR* prothrombin time international normalized ratio, *WBC* white blood cells

[a] These conditions were observed only in severe cases because of the definition of severe acute cholangitis, and therefore P values were not computed. The definition of those conditions is described in Table S1.

[b] The percentage indicates the proportion of patients with a positive finding among those for whom culture was performed.

[c] Urgent and early biliary drainage were defined as biliary drainage 6 h and 12 h after admission respectively [7].

[d] The percentage indicates the proportion of patients among those who received biliary drainage.

[e] The percentage indicates the proportion of patients among those who received biliary drainage with a plastic stent or nasobiliary catheter.

that for WBC ($P < 0.0001$) and that for CRP ($P < 0.0001$). However, The AUC for procalcitonin for moderate/severe acute cholangitis was not significantly different from that for WBC ($P = 0.78$) and that for CRP ($P = 0.29$). On the basis of the Youden index, the optimal cutoff values for procalcitonin were 2.2 ng/mL for prediction of severe acute cholangitis (sensitivity 0.97; specificity 0.73; accuracy 0.76) and 0.9 ng/mL for prediction of moderate/severe acute cholangitis (sensitivity 0.68; specificity 0.69; accuracy 0.69). The AUC for procalcitonin for severe acute cholangitis was greater than the AUC for WBC or CRP in cholangitis due to either stent occlusion or choledocolithiasis, although the comparison in the former case did not reach statistical significance (Fig. 4).

We performed exploratory ROC analyses to examine the superiority of procalcitonin over other patient parameters and biomarkers in terms of the ability to predict severe (vs mild/moderate) or moderate/severe (vs mild) acute cholangitis (Fig. S1). The predictive ability of procalcitonin on the basis of the AUC of ROC curves was generally greater than that of other markers for any severity of acute cholangitis. Prolonged leukopenia or thrombopenia due to chemotherapies might have biased the diagnosis of the severity of acute cholangitis, and therefore we excluded patients with a platelet count less than 100,000/mm^3 or a WBC count less than 4000/mm^3 due to chemotherapy. In addition, we performed sensitivity analyses in which patients who received other chemotherapies were excluded, but the results did not substantially change (data not shown). Furthermore, our sensitivity analyses excluding patients receiving each of the anti-inflammatory medications (steroid,

Table 2 Results of blood and bile cultures in patients with acute cholangitis

Species	Blood culture ($n = 170$)	Bile culture ($n = 189$)
Aerobic bacteria		
Escherichia coli	25 (43%)	71 (41%)
Klebsiella pneumoniae	12 (21%)	49 (28%)
Klebsiella oxytoca	5 (8.6%)	26 (15%)
Pseudomonas aeruginosa	5 (8.6%)	20 (11%)
Enterococcus faecium	4 (6.9%)	19 (11%)
Citrobacter freundii	3 (5.2%)	21 (12%)
Enterococcus faecalis	2 (3.4%)	31 (18%)
Enterococcus casseliflavus	2 (3.4%)	16 (9.2%)
Aeromonas sp.	2 (3.4%)	7 (4.0%)
Enterobacter cloacae	1 (1.7%)	20 (11%)
Enterobacter aerogenes	1 (1.7%)	7 (4.0%)
Enterobacter sp.	1 (1.7%)	3 (1.7%)
Streptococcus anginosus	1 (1.7%)	2 (1.1%)
Acinetobacter sp.	1 (1.7%)	1 (0.6%)
Aeromonas hydrophila	1 (1.7%)	1 (0.6%)
Morganella morganii	1 (1.7%)	1 (0.6%)
Streptococcus sp.	1 (1.7%)	1 (0.6%)
Streptococcus bovis	1 (1.7%)	
Aeromonas caviae		4 (2.3%)
Enterococcus avium		4 (2.3%)
α-Hemolytic streptococci		3 (1.7%)
Enterococcus gallinarum		2 (1.1%)
Staphylococcus aureus		2 (1.1%)
Aeromonas sobria		1 (0.6%)
Bacillus sp.		1 (0.6%)
Enterobacter agglomerans		1 (0.6%)
Serratia marcescens		1 (0.6%)
Staphylococcus epidermis		1 (0.6%)
Streptococcus agalactiae		1 (0.6%)
Streptococcus salivarius		1 (0.6%)
Anaerobic bacteria		
Clostridium perfringens	1 (2%)	9 (5.2%)
Bacteroides fragilis	1 (2%)	5 (2.9%)
Bacteroides thetaiotaomicron		1 (0.6%)
Edwardsiella tarda		1 (0.6%)
Lactobacillus sp.		1 (0.6%)
Proteus vulgaris		1 (0.6%)
Other bacteria		
Gram-positive rod (unspecified)		1 (0.6%)
Gram-negative rod (unspecified)		1 (0.6%)
Positive findings[a]	58 (34%)	174 (92%)
Number of species identified		
1	46 (79%)	59 (34%)
2	11 (19%)	65 (37%)
≥3	1 (1.7%)	50 (29%)

Values are expressed as the number and percentage in *parentheses*. The percentage indicates the proportion of patients among those who had positive blood culture or bile culture unless otherwise indicated. Two sets of blood cultures were performed for 170 patients, and bile culture was performed for 189 patients. Aerobic and anaerobic bacteria were targeted in both blood and bile cultures

[a] The percentage indicates the proportion of patients with a positive finding among those for whom culture was performed

Table 3 Severity of acute cholangitis and procalcitonin level according to the results of blood and bile cultures

	No. of cases	Severity of acute cholangitis		Procalcitonin (ng/mL)
		Moderate/severe	Severe	
Blood culture	170	87	25	
Positive	58	36 (41%)	17 (68%)	4.71 (0.87–16.96)
Negative	112	51 (59%)	8 (32%)	0.65 (0.25–2.26)
P		0.052	0.0002	<0.0001
Gram-positive cocci positive	11	7 (8.0%)	3 (12%)	4.13 (1.24–13.61)
Gram-negative rod positive	53	32 (37%)	15 (60%)	5.29 (0.86–15.73)
P		1.00	1.00	0.92
Only Gram-positive cocci positive	5	4 (4.6%)	2 (8.0%)	2.24 (0.86–20.71)
Only Gram-negative rod positive	47	29 (33%)	14 (56%)	5.29 (0.84–17.39)
P		0.64	0.64	0.83
Aerobic bacteria positive	57	35 (40%)	16 (64%)	4.13 (0.86–15.46)
Anaerobic bacteria positive	2	2 (2.3%)	1 (4.0%)	40.80 (13.49–68.10)
P		0.52	0.50	0.12
Only aerobic bacteria positive	56	34 (39%)	16 (64%)	4.10 (0.85–16.14)
Only anaerobic bacteria positive	1	1 (1.1%)	1 (4.0%)	68.10
P		1.00	0.30	0.14
Number of species				
1	46	29 (33%)	15 (60%)	3.65 (0.57–21.32)
≥2	12	7 (8.0%)	2 (8.0%)	6.83 (3.89–13.58)
P		0.77	0.28	0.0003
Bile culture		97	27	
Positive	174	92 (95%)	27 (100%)	1.40 (0.35–6.34)
Negative	15	5 (5.2%)	0	0.36 (0.18–0.50)
P		0.18	0.13	0.0017
Gram-positive cocci positive	83	42 (43%)	11 (41%)	0.86 (0.34–4.13)
Gram-negative rod positive	158	84 (87%)	25 (93%)	1.58 (0.35–8.37)
P		0.79	0.71	0.31
Only Gram-positive cocci positive	15	7 (7.2%)	2 (7.4%)	0.43 (0.23–2.11)
Only Gram-negative rod positive	90	49 (51%)	16 (59%)	1.79 (0.35–10.71)
P		0.59	1.00	0.12
Aerobic bacteria positive	174	92 (95%)	27 (100%)	1.40 (0.35–6.34)
Anaerobic bacteria positive	17	14 (14%)	4 (15%)	4.13 (1.00–22.33)
P		0.022	0.49	0.045
Only aerobic bacteria positive	157	78 (80%)	23 (85%)	0.99 (0.34–5.28)
Only anaerobic bacteria positive	0	0	0	–
P	–	–	–	–
Number of species				
1	59	32 (33%)	11 (41%)	0.96 (0.31–4.2)
2	65	26 (27%)	9 (33%)	0.99 (0.29–10.76)
≥3	50	34 (35%)	7 (26%)	1.66 (0.43-6.07)
P for trend		0.19	0.49	0.78

Values are expressed as the number and percentage in *parentheses* or the median and interquartile range in *parentheses*. The percentage indicates the proportion of patients with a specific finding among each severity group

acetaminophen, or nonsteroidal anti-inflammatory drug) or immunosuppressive drugs yielded similar results (data not shown). From analysis of positive blood culture as a surrogate of severe acute cholangitis, procalcitonin tended to be associated with a larger AUC in an ROC analysis compared with WBC or CRP (Fig. S2).

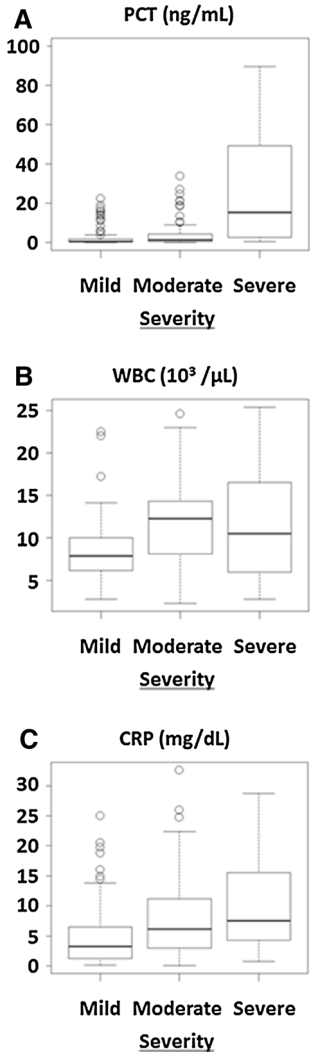

Fig. 2 Box plots of biomarker levels according to severity of acute cholangitis: **a** procalcitonin (*PCT*), **b** white blood cells (*WBC*), **c** C-reactive protein (*CRP*). The trend was assessed by the Jonckheere-Terpstra test (all $P < 0.0001$). The *bold lines* indicate the median values of the biomarkers, and the *boxes* indicate the interquartile range. *Circles* indicate outliers. Three outliers for severe acute cholangitis were outside the graph plots and thus were not plotted for procalcitonin.

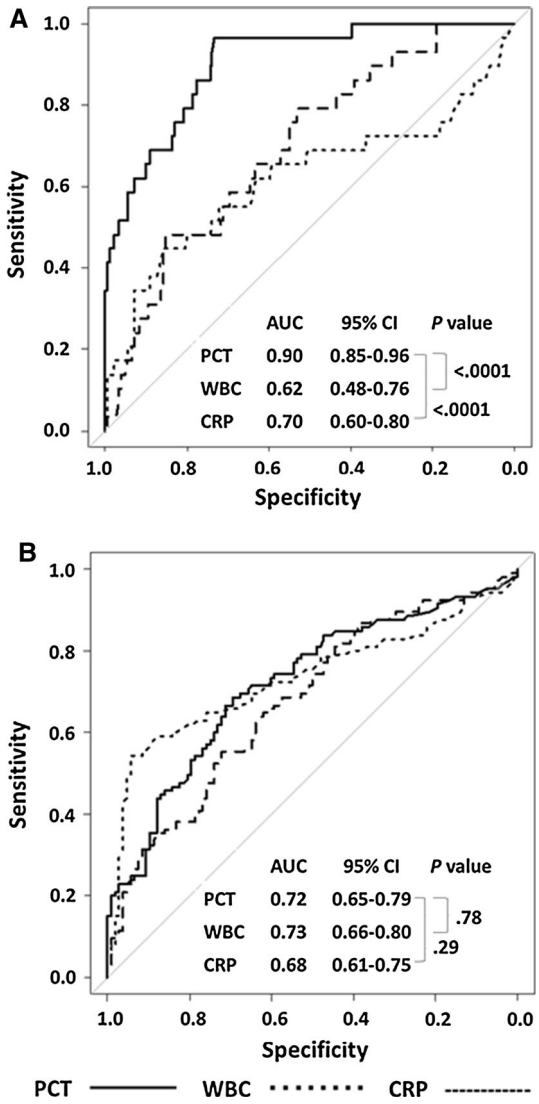

Fig. 3 Receiver operating characteristic curves for procalcitonin (*PCT*), white blood cells (*WBC*), and C-reactive protein (*CRP*) for prediction of the severity of acute cholangitis. The *P* value for comparison of the area under the curve (*AUC*) was computed by the Delong test. **a** Severe (as an outcome variable) versus mild/moderate acute cholangitis. **b** Severe/moderate (as an outcome variable) versus mild acute cholangitis. *CI* confidence interval

Risk of severe acute cholangitis according to procalcitonin level

A restricted cubic spline curve of the odds ratio of severe acute cholangitis according to the procalcitonin level with adjustment for the cause of acute cholangitis is illustrated in Fig. S3. The test for the overall relationship between procalcitonin level and severe acute cholangitis was statistically significant ($P < 0.0001$). The test for a nonlinear relationship was not statistically significant ($P = 0.62$), and the odds ratio appeared to increase linearly according to the procalcitonin level.

Discussion

In this prospective study of 213 acute cholangitis cases, we found superior ability of procalcitonin level to predict severe acute cholangitis, but this was so for moderate acute cholangitis. The serum procalcitonin level increased significantly according to the severity of acute cholangitis. In the ROC analyses, procalcitonin was shown to discriminate severe acute cholangitis significantly better than WBC or CRP. Therefore, the measurement of serum procalcitonin level for diagnosis of acute cholangitis on admission might

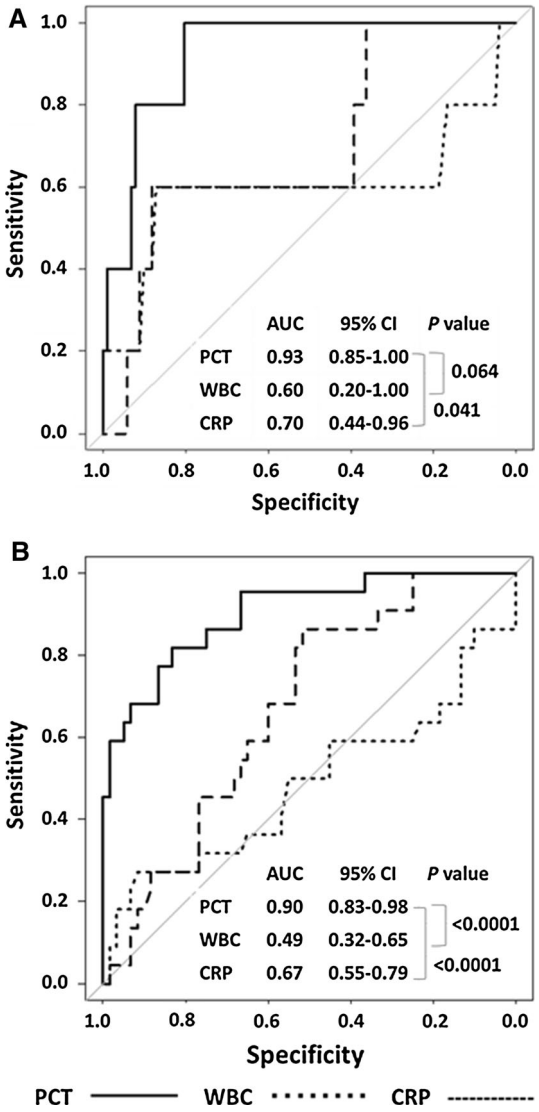

Fig. 4 Receiver operating characteristic curves for procalcitonin (*PCT*), white blood cells (*WBC*), and C-reactive protein (*CRP*) for prediction of severe acute cholangitis (as an outcome variable, vs mild/moderate acute cholangitis) according to the causes. The *P* value for comparison of the area under the curve (*AUC*) was computed by the Delong test. **a** Acute cholangitis due to stent occlusion ($n = 107$). **b** Acute cholangitis due to choledocholithiasis ($n = 82$). *CI* confidence interval

facilitate early identification of patients with severe acute cholangitis who require urgent biliary drainage.

Accumulating evidence suggests that procalcitonin is a sensitive and specific biomarker for systemic bacterial or fungal infection, whereas conventional biomarkers such as WBC and CRP are affected by noninfectious inflammatory diseases [12, 15, 26]. Another advantage of procalcitonin in this setting lies in the rapid increase of its serum level at a very early stage of disease onset compared with the levels of other biomarkers [8–11]. The usefulness of procalcitonin for early diagnosis and risk stratification of bacterial infection has been extensively examined in respiratory tract infection, urinary tract infection, and severe sepsis [12–17]. Together with an early decrease in serum procalcitonin level after the control of bacterial infection, procalcitonin-guided antibiotic therapy has gained increasing popularity [21–25]. To date, however, the usefulness of procalcitonin for early severity grading of acute cholangitis has been rarely discussed [27, 28]. Since timely treatments including biliary drainage are required to improve clinical outcomes of patients with severe acute cholangitis, the identification of a sensitive readily measured biomarker to stratify patients with acute cholangitis is clinically relevant. In 2007, the Tokyo guidelines for the management of acute cholangitis and cholecystitis were developed as the first international clinical guidelines for biliary infection [29, 30]. These guidelines were revised in 2013 (TG13) so that the severity of acute cholangitis and the indication of biliary drainage could be determined solely on the basis of the findings of physical examinations and laboratory tests on admission [7, 18]. Several studies showed that the severity according to the guidelines was correlated with worse outcomes of patients with acute cholangitis [31, 32]. In a retrospective study of 110 patients, the AUC for procalcitonin, WBC, and CRP in the ROC analyses were 0.75 (95% confidence interval 0.63–0.87), 0.47 (95% confidence interval 0.33–0.61), and 0.67 (95% confidence interval 0.54–0.77) respectively for severe acute cholangitis (vs mild/moderate) [28]. Despite a well-designed study, however, this study focused only on severe cases due to choledocholithiasis, and hence a further investigation was warranted to ensure generalizability. The current study focuses on not only choledocholithiasis cases but also on all other causes, such as stent occlusion.

The current study is the first prospective study which has demonstrated that procalcitonin predicts severe acute cholangitis with better accuracy than conventional biomarkers (WBC and CRP), regardless of the cause. The optimal cutoff value for procalcitonin for prediction of severe acute cholangitis in our ROC analysis was 2.2 ng/mL, consistent with the findings of a previous study [28]. It is also consistent with the general cutoff value for procalcitonin for prediction of severe sepsis (2.0 ng/mL) [21–25]. Despite the reduced statistical power, the results of the ROC analysis using positive blood culture as a surrogate for severe acute cholangitis were consistent with our primary hypothesis testing. Given that serum procalcitonin level can be measured within 1 h of patient admission in the current system, procalcitonin-guided risk stratification can facilitate prompt identification of patients in whom urgent biliary drainage (within 6 h) is indicated. Furthermore, there might be subsets of patients for whom procalcitonin as a prognostic factor for severe acute cholangitis is particularly useful. Patients receiving steroid therapy are less likely to

have high temperature [33, 34], and serum levels of WBC and CRP might be affected by this medication. Patients with severe sepsis or elderly patients occasionally have low to normal temperature rather than high temperature [35, 36]. Given that the current study included a substantial rate of patients receiving antipyretic drugs and steroid therapy, procalcitonin might provide a more robust evaluation of the severity of acute cholangitis for those patients, and a prospective investigation is desired. On the other hand, the current study found no evidence of the superiority of procalcitonin for diagnosis of moderate/severe acute cholangitis. Although procalcitonin has been shown to reflect the presence and severity of systemic bacterial infection, it might be insensitive to localized infectious diseases, and thus remain at a low level [8, 37]. In those patients with a low procalcitonin level on admission, repeated measurements of serum procalcitonin level during the early time course might mitigate this problem. A prospective study taking cost-effectiveness into account is warranted to confirm the usefulness of procalcitonin to predict the severity of acute cholangitis [38].

In secondary analyses focusing on the causes of acute cholangitis, we found that procalcitonin had good ability to predict severe acute cholangitis both in acute cholangitis due to stent occlusion and in that due to choledocholithiasis. As shown in Table 1, patients with acute cholangitis due to stent occlusion were less likely to have severe acute cholangitis, presumably because those patients were likelier to have had symptoms of acute cholangitis and therefore present to our outpatient clinic early. Therefore, the usefulness of procalcitonin for prediction of severe acute cholangitis might be attenuated in acute cholangitis due to stent occlusion. In the present study, however, procalcitonin appeared to be useful to predict severe acute cholangitis regardless of the causes. Given the small number of patients in each subgroup in this study, however, further investigation is warranted to confirm the cause-specific cutoff values. In patients with the occlusion of a stent for malignant biliary obstruction, an early reintervention might be indicated regardless of the disease severity, because those patients might be immunosuppressed via chemotherapy and underlying malignancy.

Positive blood culture was associated with severe acute cholangitis, suggesting a potential of this finding as a surrogate for the disease severity. Although the small sample sizes precluded a robust statistical evaluation, there were no significant associations between the types and numbers of the bacteria identified and severe acute cholangitis. Nonetheless, the number of bacteria identified by blood and bile cultures, positive blood culture, and positive anaerobic bacteria culture tended to be associated with a higher level of procalcitonin, which potentially suggests the disease severity. In addition, multiple bacteria in bile culture were more frequently observed in stent occlusion cases than in choledocholithiasis cases, suggesting the possibility of bacterial contamination in patients with prolonged placement of biliary stents. Therefore, further investigation is warranted to examine the predictive abilities of these findings for severe acute cholangitis.

Several limitations should be acknowledged in this study. Although this study was based on a prospective design, the relatively small sample size might have precluded a robust statistical evaluation for some secondary analyses. Another limitation was that data were collected in a single high-volume center, and the characteristics of the enrolled patients might be biased (e.g., causes of and pathogens in acute cholangitis). In the ROC analysis for positive blood culture as a secondary analysis, culture was not obtained in all cases. Parameters other than WBC count and CRP level evaluated in this study might be useful for diagnosis of severe acute cholangitis [39, 40]. Therefore, a prospective study including a large number of patients from hospitals in various settings is warranted to validate the usefulness of procalcitonin for prediction of the severity of acute cholangitis.

In conclusion, the serum level of procalcitonin well predicted severe acute cholangitis on patient admission compared with conventional biomarkers. Our findings suggest potential usefulness of procalcitonin-based risk stratification of acute cholangitis to facilitate timely identification of patients in whom biliary drainage is indicated. A large prospective study including a cost-effective analysis is warranted.

Compliance with ethical standards

Conflict of interest Hiroyuki Isayama has received a research grant and a speaker fees from Thermo Fisher Scientific Inc. This study was not funded by Thermo Fisher Scientific Inc. The other authors declare that they have no conflict of interest.

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