Predictive Factors and Microbial Spectrum for Infectious Complications after Hepatectomy with Cholangiojejunostomy in Perihilar Cholangiocarcinoma

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Abstract

Background: Despite advances in surgical techniques and peri-operative management, post-operative infectious complications still are common after perihilar cholangiocarcinoma (PHCC). This study investigated the predictive factors and microbial spectrum for infections after hepatectomy with cholangiojejunostomy performed to treat PHCC.

Methods: A total of 70 consecutive patients, who underwent hepatectomy with cholangiojejunostomy by the same surgeons at a tertiary referral medical center between September 2010 and January 2019, were enrolled. Clinical data were reviewed for multivariable analysis to find independent risk factors for infectious complications. Microorganisms isolated from bile and infection sites were counted to explore the microbial spectrum.

Results: A total of 43 patients (61.4%) suffered post-operative infections (33 with surgical site infection [SSI], four with bacteremia, three with pneumonia, 10 with cholangitis, and two with fungus infectious stomatitis), and 28 of them (65.1%) had a positive bile culture. Four independent risk factors were identified: male sex (odds ratio [OR] 12.737; 95% confidence interval [CI] 2.298–70.611; $p=0.004$), red blood cell (RBC) count $<3.8 \times 10^{12}/L$ (OR 5.085; 95% CI 1.279–20.211; $p=0.021$), total cholesterol (TC) <2.90 mmol/L (OR 5.715; 95% CI 1.534–21.299; $p=0.009$), and serum Na^+ >145 mmol/L (OR 10.387; 95% CI 1.559–69.201; $p=0.016$) on post-operative day (POD) 1. A total of 217 and 196 microorganisms were cultured from 311 and 627 specimens, respectively, collected from pre-/intra-operative bile and possible infection sites. *Staphylococcus*, *Enterococcus*, *Acinetobacter*, *Streptococcus*, and *Escherichia* were the most common findings of bile culture. The first five organisms most frequently isolated from infection sites were *Enterococcus*, *Staphylococcus*, *Klebsiella*, *Acinetobacter*, and *Candida*. A total of 18 patients (64.3%) had at least one species isolated from infection sites that had appeared in a previous bile culture.

Conclusions: Male sex, erythrocytopenia, hypocholesterolemia, and hypernatremia on POD 1 are independent risk factors for infectious complications. For patients without positive bile cultures, third-generation cephalosporins could be considered as the prophylactic antibiotic. It is important to monitor the pathogens throughout the hospital stay.

Keywords: cholangiojejunostomy; hepatectomy; Klatskin tumor; post-operative infections; risk factors

PERIHILAR CHOLANGIOCARCINOMA (PHCC) accounts for about 50%–60% of all cholangiocarcinomas, with a manifoldly higher incidence in the East than in the West [1]. By definition, PHCC abuts or invades the central part of the liver, so it is necessary for patients to undergo hepatectomy with cholangiojejunostomy to achieve long-term survival and

decreased hepatic recurrence [2,3]. According to a series of studies performed at Nagoya University [4–6], despite improvements in care, the incidence of infectious complications after such major operations is still as high as 28.3%–41.9%, which may have interactive relations with other complications, such as bile leakage, pancreatic and chylous fistula

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formation, etc. A World Health Organization survey [7] also pointed out that hospital-acquired infections (HAI), especially surgical site infections (SSIs), affect many patients and impose heavy endemic burdens in developing countries. In this context, early detection of post-operative infectious complications is meaningful.

In the present study, we reviewed clinical data (demography, peri-operative management, surgical procedure, and pre-operative/early post-operative laboratory data) of patients with PHCC admitted to our center to investigate the predictive factors and microbial spectrum of infectious complications in patients with PHCC who had undergone hepatectomy with cholangiojejunostomy. Our goal was to provide bases for early intervention.

Patients and Methods

Patients

This retrospective study was conducted on 70 consecutive patients with PHCC undergoing hepatectomy with cholangiojejunostomy by the same group of surgeons at a tertiary referral medical center in Nanjing, China, from September 2010 to January 2019. The study was approved by the institutional research review committee.

Pre-operative management

All disease was diagnosed by a multidisciplinary team composed of surgeons, oncologists, radiologists, ultrasonologists, and pathologists. Treatment decisions, including whether to perform pre-operative biliary drainage (PBD), also were made by the team.

The criteria for PBD in our center are as follows: (1) Serum total bilirubin (TB) ≥ 170 $\mu\text{mol/L}$ (10 mg/dL); (2) preserved liver volume (based on CT volume measurement or three-dimensional reconstruction when TB < 51 $\mu\text{mol/L}$ or 3 mg/dL) $< 40\%$ or indocyanin green (ICG) K-F [8] 0.05 (when TB < 34 $\mu\text{mol/L}$ or 2 mg/dL) requiring portal vein embolization (PVE); (3) pre-operative cholangitis; (4) scheduled extensive hepatectomy (four or more segments) combined vascular resection and reconstruction and/or pancreaticoduodenectomy (PD); or (5) severe malnutrition.

Percutaneous transhepatic biliary drainage (PTBD) was the major drainage method before 2014. After that, priority was given to endoscopic nasobiliary drainage (ENBD) with or without endoscopic biliary stenting (EBS) because one patient suffered PTBD catheter tract recurrence. All patients underwent bile reinfusion through a naso-intestinal feeding tube or by oral administration even if there were bacteria in the bile. For bile drained externally from the catheter, biochemical examination, along with bacterial smear and culture, were performed at least once a week before surgery.

Nutrition screening was carried out on all patients on admission by Nutritional Risk Screening (NRS-2002) [9], Scored Patient-Generated Subjective Global Assessment (PG-SGA) [10], and performance status (PS) assessment [11]. A bioelectrical impedance analysis (BIA) system was added to the nutrition assessment system after 2013. For patients with nutritional risk (NRS score ≥ 3 , PG-SGA score ≥ 4 , or low skeletal muscle mass suggested by the BIA system), enteral nutrition (EN), usually as the first choice for nutritional support, was delivered on the basis of regular diets. Every week during the pre-operative

period, patients were re-evaluated to determine appropriate support measures. Synbiotic treatment was applied as a routine on the basis of the results of previous randomized controlled trials [12,13].

If patients suffered pre-operative infections (cholangitis, bacteremia, etc.), surgery was postponed until the infection was well controlled. Intravenous drip of prophylactic antibiotic 30 min before surgery was a regular practice. Depending on the half-life of the antibiotic and the intra-operative blood loss (≥ 1500 mL), an additional dose might be considered during the operation. Third-generation cephalosporins were used in patients without positive bile cultures. For patients with positive cultures, antibiotics were selected according to the drug susceptibility results. Prophylactic antibiotics continued until 48 hours after surgery.

Surgery

The operations were performed by the same group of surgeons after achieving TB < 51 $\mu\text{mol/L}$ (3 mg/dL) and a PS score < 2 . Patients at high risk of seeding metastasis, defined as those having an elevated serum CA125 concentration, suspected celiac nodules or effusion suggested by imaging, or nodular neoplasms with unilateral hepatic pedicle invasion underwent laparoscopic exploration in advance to determine the likely value of a resection. The scope of the resection of the liver and the value of combining its excision with more aggressive procedures were based on pre-operative evaluation and intra-operative exploration. Specimens were resected en bloc, and the bile duct was the last to be cut off. All patients had extra-hepatic choledochectomy, regional lymph node resection, distal lymph node biopsies (Groups 9 and 16), and Roux-en-Y cholangiojejunostomy. Bile or the catheter head was cultured intra-operatively. Nutritional jejunostomy was received by the majority patients for post-operative EN. Rapid pathologic examination of the surgical margins was required. The abdominal cavity was drained by grooved drainage tubes placed close to the liver section and anastomosis.

Post-operative management

Multimode analgesia was applied to patients after surgery. Nutritional support measures were consistent with previous practice and adjusted according to weekly evaluation.

Fluid from the abdominal drains was examined on PODs 1, 3, and 7, including biochemical examination, bacterial smear, and culture. Ascites examination was maintained at least once a week until drainage tube removal. In patients with thoracentesis, hydrothorax culture was performed immediately after the procedure and repeated at least once a week for surveillance. If a febrile patient developed shivers or a body temperature exceeding 38.5°C , aerobic and anaerobic blood cultures were conducted. Secretions were cultured when suspicious discharge was observed in the incision or other sites. Both the diagnosis of infectious complications and the use of antibiotics were determined by clinicians and clinical pharmacists, based on clinical features, positive etiologic results, and other laboratory or radiology tests.

Recording of peri-operative complications

Infectious complications were recorded for as long as 30 days after the surgery. The definition of SSI was consistent

with the previous rule [7]. Cholangitis was diagnosed according to the Tokyo Guidelines [14]. For post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis (PEP), the criteria established by the Japanese Society of Gastroenterology [15] were followed. The definitions and grading systems established by the International Study Group of Liver Surgery (ISGLS) were used to make definite diagnoses of bile leakage [16] and liver failure [17]. Diagnoses of post-operative pancreatic [18] and chylous [19] fistula were in reference to the International Study Group on Pancreatic Surgery (ISGPS). The definition of chylous fistula also was applied to patients without PD. Acute kidney injury was defined and graded by the Kidney Disease Improving Global Outcomes (KDIGO) organization [20]. The definitions of bacteremia and pneumonia were the same as those at Nagoya University [4]. The severity of all complications was assessed by the Clavien-Dindo classification [21].

Statistical analysis

All statistical analyses were performed with SPSS software Version 20.0 (SPSS, Chicago, IL). Quantitative data subject to a normal distribution were expressed as mean $\pm$ standard deviation (SD) and compared by the Student *t*-test. Categorical variables were shown as numbers and compared using the χ^2 test or Fisher exact test. Independent risk factors were analyzed by multivariable binary logistic regression analysis with a threshold of $p < 0.10$. A result was considered statistically significant when $p < 0.05$.

Results

Patient demographics

The characteristics and operative procedures of patients are shown in Table 1. A total of 70 consecutive patients were

TABLE 1. CHARACTERISTICS AND OPERATIVE PROCEDURES

	With infection (N=43)		Without infection (N=27)		p
Mean age ($\pm$ standard deviation) (y)	61	± 10	57	± 11	0.699
Male (%)	34	(79.1)	14	(51.9)	0.017
Pre-operative co-morbidities (%)					
Malignant tumor history	4	(9.3)	2	(7.4)	1.000
Hypertension	9	(20.9)	7	(25.9)	0.628
Diabetes	6	(14.0)	1	(3.7)	0.326
Cholelithiasis	8	(18.6)	5	(18.5)	0.993
Chronic hepatitis B	6	(14.0)	1	(3.7)	0.326
Bismuth type (I/II/IIIa/IIIb/IV)	2/7/12/10/12		0/6/5/8/8		0.532
Nutritional screening on admission					
Body Mass Index (kg/m ²)	22.6	± 3.4	23.6	± 3.3	0.721
PS score (0/1/2)	30/10/3		21/6/0		0.215
NRS score ≥ 3 (%)	25	(58.1)	10	(37.0)	0.086
PG-SGA score ≥ 4 (%)	25	(58.1)	12	(44.4)	0.264
Pre-operative biliary drainage (without/PTBD/ENBD/ENBD+EBS/surgical)	10/15/15/3/0		7/3/15/1/1		0.091
Pre-operative PVE (%)	6	(14.0)	4	(14.8)	1.000
Positive bile culture (%)	28	(65.1)	14	(51.9)	0.270
With MDR	14	(32.6)	8	(29.6)	0.797
Pre-operative cholangitis (%)	11	(25.6)	4	(14.8)	0.285
PEP (%)	2	(11.1; N=18)	1	(8.8; N=16)	1.000
ICG-K	0.189 $\pm$ 0.042		0.191 $\pm$ 0.057		0.163
CEA (0–10, ng/ml) >10 (%)	4	(9.3)	3	(11.1)	1.000
CA19-9 (0–27, U/ml) >27 (%)	37	(86.0)	24	(88.9)	1.000
ASA class >2(%)	33	(76.7)	17	(63.0)	0.214
Type of hepatectomy (%)					
(Extended)Left hemihepatectomy	16	(37.2)	13	(48.1)	0.366
(Extended)Right hemihepatectomy	14	(32.6)	5	(18.5)	0.199
Left trisectionectomy	4	(9.3)	2	(7.4)	1.000
Right trisectionectomy	4	(9.3)	4	(14.8)	0.749
Other (<4 segments)	5	(11.6)	3	(11.4)	1.000
Combined with PD (%)	4	(9.3)	2	(7.4)	1.000
Combined with PV and/or HA resection (%)	15	(34.9)	5	(18.5)	0.140
Combined with nutritional jejunostomy (%)	29	(67.4)	20	(74.1)	0.556
Choledochoenteric anastomoses ≥ 2 (%)	16	(37.2)	8	(29.6)	0.515
Operative time (min)	634	± 117	604	± 80	0.131
Estimated blood loss (mL) >1,100 (%)	10	(23.3)	10	(37.0)	0.214
Perioperative blood transfusion (%)	36	(83.7)	19	(70.4)	0.185

ASA=American Society of Anesthesiologists; BMI=Body Mass Index; CA=carbohydrate antigen; CEA=carcinoembryonic antigen; EBS=endoscopic biliary stenting; ENBD=endoscopic nasobiliary drainage; ERCP=endoscopic retrograde cholangiopancreatography; ICG=indocyanine green; NRS=Nutritional Risk Screening; PD=pancreaticoduodenectomy; PEP=post-ERCP pancreatitis; PG-SGA=Patient-Generated Subjective Global Assessment; PS=performance status; PTBD=percutaneous transhepatic biliary drainage, PVE=portal vein embolization.

TABLE 2. LABORATORY RESULTS

Pre-operative/POD 1 (%)	With infection (N=43)	Without infection (N=27)	p
WBC ($3.5-9.5 \times 10^9/L$) >9.5	2 (4.7)/33 (76.7)	0/21 (77.8)	0.519/0.920
RBC ($3.8-5.1 \times 10^{12}/L$) <3.8	9 (20.9)/27 (62.8)	3 (11.1)/ 9 (33.3)	0.462/0.016
Hb (115–150 g/L) $<120/<100$	16 (37.2)/10 (23.3)	13 (48.1)/ 8 (29.6)	0.366/0.553
PLR ≥ 150	22 (51.2)/38 (88.4)	16 (64.0)/26 (96.3)	0.304/0.475
GPT (5–40, U/L) $\geq 80/\geq 200$	13 (30.2)/36 (83.7)	10 (37.0)/22 (81.5)	0.555/1.000
GOT (8–40, U/L) $\geq 80/\geq 200$	8 (18.6)/36 (83.7)	8 (29.6)/23 (85.2)	0.285/1.000
AKP (47–185 U/L) ≥ 185	21 (48.8)/ 8 (18.6)	15 (55.6)/ 7 (25.9)	0.584/0.467
GGT (7–35 U/L) ≥ 35	40 (93.0)/39 (90.7)	24 (88.9)/25 (92.6)	0.871/1.000
TB (5–20.5 $\mu\text{mol/L}$) ≥ 34	16 (37.2)/27 (62.8)	13 (48.1)/17 (63.0)	0.366/0.988
Albumin (40–55 g/L) <35	6 (14.0)/41 (95.3)	4 (14.8)/25 (92.6)	1.000/1.000
TC (2.90–5.72 mmol/L) <2.90	2 (4.7)/35 (81.4)	0/12 (44.4)	0.519/0.001
Na ⁺ (135–145 mmol/L) >145	1 (2.3)/18 (41.9)	0/ 5 (18.5)	1.000/0.043
CRP (0–8 mg/L) >8	8 (18.6)/41 (95.3)	1 (3.7)/27 (100.0)	0.148/0.519
Prealbumin (150–380 mg/L) <150	4 (9.3)/20 (46.5)	1 (3.7)/ 7 (25.9)	0.683/0.085
PT (10–15 s) >15	0/22 (51.2)	0/10 (37.0)	–/0.248

AKP=alkaline phosphatase; CRP=C-reactive protein; GGT=glutamyl transpeptidase; GOT=glutamic oxaloacetic transaminase; GPT=glutamic pyruvic transaminase; Hb=hemoglobin; PLR=platelet-to-lymphocyte ratio; POD=post-operative day; PT=prothrombin time; RBC=red blood cells; SC=serum cholinesterase; TB=total bilirubin; TC=total cholesterol; WBC=white blood cells.

enrolled, comprising 48 males (68.6%) and 22 females. The mean age was 60 years (range 36–81 years). Bismuth type III/IV PHCC accounted for 78.6% of all patients. Six patients (8.6%) had a malignant tumor history. Hypertension and diabetes mellitus were found in 16 (22.9%) and 7 (10.0%), respectively. There were 13 patients (18.6%) and 7 patients (10.0%) who had cholelithiasis and chronic hepatitis B, respectively.

A series of 53 patients (75.7%) received PBD, and 10 of them (18.9%) underwent PVE at the same time. The methods of biliary drainage were PTBD in 18 patients (34.0%), ENBD in 30 (56.6%), ENBD combined with EBS in 4 (5.7%), and surgical drainage in 1 (1.4%). Cholangitis and PEP occurred in 15 (21.4%) and 3 (8.8%; n=34) patients, respectively. All patients received appropriate conservative treatment without effect on the scheduled surgery. The average pre-operative hospital stay was 10 days (range 4–34 days) and 32 days (range 4–68 days) in patients with or without PBD, respectively ($p < 0.05$).

Approximately three fourths of the patients (71.4%) had an ASA grade >2 . Extensive hepatectomy was performed in 62 patients (88.6%). Twenty patients (28.6%) underwent portal vein or hepatic artery or both resection and reconstruction. One patient had inferior vena cava resection and reconstruction. A PD was performed in 6 patients (8.6%), and 49 (70.0%) patients underwent nutritional jejunostomy. Another 24 patients (34.3%) had two or more choledochenteric anastomoses created. The mean operation time was 622 minutes (range 390–940 minutes). The mean estimated blood loss was 1,158 mL (range 200–4,700 mL). The rate of peri-operative blood transfusion was 78.6%.

Laboratory results are listed in Table 2. Pre-operative tests were performed within three days before surgery. The incidences of erythrocytopenia, hypocholesterolemia, and hypernatremia on POD 1 were higher in patients suffering post-operative infectious complications ($p < 0.05$).

TABLE 3. POST-OPERATIVE OUTCOMES

	With infection (N=43)	Without infection (N=27)	p
Infectious complication (%)	43 (100.0)	–	–
SSI	33 (76.7)	–	–
Superficial/deep incisional	4 (9.3)	–	–
Organ/space	33 (76.7)	–	–
Bacteremia	4 (9.3)	–	–
Pneumonia	3 (7.0)	–	–
Cholangitis	10 (23.3)	–	–
Fungus infectious stomatitis	2 (4.7)	–	–
Bile leakage (grade A/B/C) (%)	7/9/2	1/2/0	0.682
POPF (%) (biochemical fistula/grade B/C)	1/2/1	0	–
Chylous fistula (%)	3 (7.0)	4 (14.8)	0.513
Hepatic failure (grade A/B/C) (%)	0/2/1	1/1/1	0.422
AKI (KDIGO grade 3) (%)	3 (7.0)	0	0.426
Intra-abdominal bleeding, (%)	2 (4.7)	0	0.519
Clavien-Dindo grade $\geq$ IIIa (%)	23 (53.5)	3 (11.1)	0.000
Hospital death (%)	2 (4.7)	0	0.519
Mean post-operative hospital stays (d)	36 $\pm$ 19	24 $\pm$ 11	0.009

AKI=acute kidney injury; POPF=post-operative pancreatic fistula; SSI=surgical site infection.

TABLE 4. MULTIVARIABLE ANALYSES FOR RISK FACTORS FOR POST-OPERATIVE INFECTIOUS COMPLICATIONS

Variable	β	OR	95% CI	p
Male sex	2.545	12.737	2.298–70.611	0.004
POD 1 RBC $<3.8 \times 10^{12}/L$	1.626	5.085	1.279–20.211	0.021
POD 1 TC <2.90 mmol/L	1.743	5.715	1.534–21.299	0.009
POD 1 $Na^+ >145$ mmol/L	2.341	10.387	1.559–69.201	0.016
With PBD	–	–	–	0.880
NRS score ≥ 3 on admission	–	–	–	0.835
POD 1 prealbumin <150 mg/L	–	–	–	0.767

CI=confidence interval; NRS=Nutritional Risk Screening; OR=odds ratio; PBD=pre-operative biliary drainage; POD=post-operative day; RBC=red blood cells; TC=total cholesterol.

Post-operative outcomes and risk factors for infectious complications

Post-operative outcomes are listed in Table 3. According to the Clavien-Dindo classification, 26 patients (37.1%) suffered severe complications (Grade IIIa or higher). Of the two hospital deaths, one patient died of septic shock, and hepatic failure caused the other. In this cohort, infectious complications, consisting of SSI, bacteremia, pneumonia, cholangitis, and fungus infectious stomatitis occurred in 43 patients (61.2%). Patients with post-operative infectious complications had longer hospital stays, a higher incidence of bile leakage, and more serious complications ($p < 0.05$).

Several potential risk factors for post-operative infectious complications were examined by multivariable analysis (Table 4). Four independent factors were found: Male sex (OR 12.737; 95% CI 2.298–70.611; $p = 0.004$), RBC count $<3.8 \times 10^{12}/L$ (OR 5.085; 95% CI 1.279–20.211; $p = 0.021$), TC <2.90 mmol/L (OR 5.715; 95% CI 1.534–21.299; $p = 0.009$), and $Na^+ >145$ mmol/L (OR 10.387; 95% CI 1.559–69.201; $p = 0.016$) on POD 1. The relation between the number of risk factors and morbidity is shown in Table 5.

Pre-operative or intra-operative bile and blood cultures

Forty-two patients (79.2%) with PBD had a positive bile culture, and 30 of them (71.4%) had a mixed infection. There were 217 microorganisms isolated from 311 bile specimens (Table 6). Twenty-five microorganisms were cultured from 71 specimens intra-operatively. The most frequent microorganism was *Staphylococcus* spp., followed by *Enterococcus* spp., *Acinetobacter* spp., *Streptococcus* spp., and *Escherichia coli* in descending order of frequency. Multi-drug-resistant (MDR) pathogens, defined as those not susceptible to at least one agent in three or more antimicrobial categories [22], were found in 55 bile specimens

from 22 patients (52.4%). Cholangitis occurred in 13 and 2 patients with or without PBD before surgery, respectively. Bacteremia was documented in three patients who had cholangitis before surgery. All microorganisms cultured from blood specimens (*Pseudomonas aeruginosa*, *E. coli*, and *Klebsiella pneumoniae*) were consistent with those from bile obtained before surgery.

Post-operative cultures from the sites of infectious complications

Almost 200 microorganisms ($n = 196$) were isolated from the 627 specimens collected at possible sites of infection in 43 patients. The microbial spectrum is shown in Table 7. The most frequent microorganisms were *Enterococcus*, *Staphylococcus*, *Klebsiella*, *Acinetobacter*, and *Candida*. From the 28 patients (65.1%) who had positive bile cultures before surgery, 161 microorganisms were isolated from 410 specimens. *Enterococcus*,

TABLE 6. MICROORGANISMS CULTURED FROM BILE IN PATIENTS HAVING PRE-OPERATIVE BILIARY DRAINAGE

Species (%)	No. of patients positive (N=42)	No. of positive specimens (N=217)
Main gram-positive bacteria		
<i>Staphylococcus</i>	20 (47.6)	38 (17.4)
<i>Enterococcus</i>	11 (26.2)	36 (16.5)
<i>Streptococcus</i>	9 (21.4)	22 (10.1)
<i>Bacillus</i>	3 (7.1%)	3 (1.4)
MRSA	1 (2.4)	1 (0.5)
Main gram-negative bacteria		
<i>Acinetobacter</i>	11 (26.2)	25 (11.5)
<i>Escherichia</i>	9 (21.4)	21 (9.6)
<i>Klebsiella</i>	8 (19.1)	17 (7.8)
<i>Pseudomonas</i>	6 (14.3)	12 (5.5)
<i>Rhizobium</i>	3 (7.1)	3 (1.4)
<i>Citrobacter</i>	2 (4.8)	12 (5.5)
<i>Enterobacter</i>	2 (4.8)	8 (3.7)
<i>Aeromonas</i>	2 (4.8)	3 (1.4)
<i>Serratia</i>	1 (2.4)	2 (0.9)
<i>Xanthomonas</i>	1 (2.4)	1 (0.5)
Others ^a	7 (16.7)	8 (3.7)

MRSA=methicillin-resistant *Staphylococcus aureus*.

^a*Cupriavidus paucula*, *Micrococcus luteus*, *Vibrio alginolyticus*, *Aerococcus viridans*, *Proteus mirabilis*, *Rothia mucilaginosa*, and *Sphingomonas paucimobilis*.

TABLE 5. RELATION BETWEEN INCIDENCE OF INFECTIOUS COMPLICATIONS AND NUMBER OF RISK FACTORS

No. of risk factors	No. of patients	Number of infectious complications (%)
0	2	0
1	15	3 (20.0)
2	27	16 (59.3)
3	19	17 (89.5)
4	7	7 (100)

TABLE 7. MICROORGANISMS CULTURED FROM SITES OF INFECTIOUS COMPLICATIONS

Species (%)	Overall		With positive bile culture	
	No. of patients positive (N=43)	No. of positive specimens (N=196)	No. of patients positive (N=28)	No. of positive specimens (N=161)
Main gram-positive bacteria				
<i>Enterococcus</i>	18 (41.9)	33 (16.8)	13 (46.4)	29 (18.0)
<i>Staphylococcus</i>	18 (41.9)	25 (12.8)	12 (42.9)	15 (9.3)
<i>Bacillus</i>	4 (9.3)	5 (2.6)	4 (14.3)	5 (3.1)
MRSA	2 (4.7)	2 (1.0)	1 (3.6)	1 (0.6)
Main gram-negative bacteria				
<i>Klebsiella</i>	12 (27.9)	23 (11.7)	11 (39.3)	21 (13.0)
<i>Acinetobacter</i>	8 (18.6)	11 (5.6)	2 (7.1)	2 (1.2)
<i>Escherichia</i>	6 (14.0)	35 (17.9)	5 (17.9)	34 (21.1)
<i>Pseudomonas</i>	6 (14.0)	8 (4.1)	4 (14.3)	6 (3.7)
<i>Xanthomonas</i>	5 (11.6)	22 (11.2)	4 (14.3)	20 (12.4)
<i>Enterobacter</i>	4 (9.3)	6 (3.1)	3 (10.7)	4 (2.5)
<i>Citrobacter</i>	2 (4.7)	2 (1.0)	2 (7.1)	2 (1.2)
<i>Aeromonas</i>	2 (4.7)	2 (1.0)	0	0
<i>Candida</i>	7 (16.3)	15 (7.7)	5 (17.9)	11 (6.8)
Other ^a	3 (7.0)	7 (3.6)	3 (10.7)	7 (4.3)

MRSA = methicillin-resistant *Staphylococcus aureus*.

^a*Comamonas testosteroni*, *Sphingomonas paucimobilis*, *Elizabethkingia meningoseptica*.

Staphylococcus, *Klebsiella*, *E. coli*, and *Acinetobacter* were the most frequently isolated in patients with positive bile cultures. Eighteen patients (64.3%) had at least one species isolated from infection sites that had appeared in a previous bile culture, but only one third of them presented with both pre-operative and intra-operative bile specimens.

Discussion

Today, surgery is the only chance for patients with PHCC to achieve long-term survival. However, the incidence of post-operative infectious complications is still high according to the present and previous studies. For patients with such complications, surgery usually means a long hospital stay, delayed anti-neoplastic therapy, and a high economic burden. In this context, this study investigated predictive factors for post-operative infectious complications and the peri-operative microbial spectrum to provide bases for early detection and effective intervention.

In this study, the incidence rate of post-operative infectious complications was 61.2%, higher than in studies (28.3%–41.9%) conducted at Nagoya University [4–6]. There are several possible reasons: First, morbidity differed depending on the recording of complications, especially minor complications. Second, a considerable portion of patients in the Nagoya studies did not suffer from PHCC, which might influence the results. Third, more than half of our patients (52.4%) had MDR pathogens cultured from the bile, and this figure was eight times higher than in Nagoya (6.4%) [5], which could explain the results to some extent.

Through multivariable analysis, four independent risk factors (male sex, RBC $<3.8 \times 10^{12}/L$, TC <2.90 mmol/L, Na⁺ >145 mmol/L on POD 1) were investigated. No pre-operative risk factor was found, which may indicate the effectiveness

of the present peri-operative management performed in our center.

Erythrocytes play an important role in human innate immunity. Bacteria in the bloodstream can be attracted and kept by erythrocytes and killed by oxidation [23,24]. There is no doubt that erythrocytopenia would undermine the protective mechanism. Previous study [25] showed that peri-operative blood transfusion may lead to greater morbidity, prolonged hospital stays, and other adverse outcomes. As a limited resource, it is reasonable to reduce allogenic blood transfusion when possible. Considering the potential risk of seeding via autologous blood transfusion, peri-operative use of recombinant human erythropoietin (rhEPO) may be a useful supplement, which has been confirmed in cardiac [26], orthopedic [27], and gastrointestinal [28] surgery. In addition to the generally accepted prevention and treatment of anemia, several animal experiments [29–31] indicate that rhEPO also can promote liver regeneration, reduce ischemia–reperfusion injury, and improve survival after hepatectomy. However, there still is a lack of clinical trials to explore the value of rhEPO in hepatobiliary surgery.

The mean pre-operative and post-operative serum cholesterol concentrations were 4.68 and 2.72 mmol/L, respectively ($p < 0.05$). Multiple cumulative factors associated with surgery, inflammation, and infection may contribute to this result. Meanwhile, hypocholesterolemia could cause the development of infections by a variety of mechanisms: Declining ability of lipopolysaccharide binding and neutralizing, a decreased number of circulating lymphocytes, limited tissue repair and regeneration, insufficient steroid supply in vivo, and a dysfunctional hypothalamic–pituitary–adrenal axis [32–35]. So hypocholesterolemia and infection interact as both cause and effect. Lee et al. [35] found that the increase of serum cholesterol was related to a better prognosis in patients undergoing gastrointestinal surgery, suggesting the potential value of cholesterol in surveillance and therapy.

Some studies [36,37] have pointed out that changing the content of branched-chain amino acids and the source of lipids in parenteral nutrition could have an effect on serum cholesterol. The specific mechanism has not been clarified yet. However, there is no related report on the value of these measures in EN.

The relation between hypernatremia and adverse clinical outcomes has been reported several times [38–40]. In the present study, it was found that hypernatremia was an independent risk factor for post-operative infectious complications, as was found by previous investigators [41]. Hypernatremia creates cellular dehydration and promotes peripheral insulin resistance, aggravating metabolic and immune disturbances, which might be the mechanism of development and exacerbation of infection [42,43]. Although the rate of hypernatremia relief still is controversial [44,45], it is suggested that patients can benefit from early identification and correction of post-operative hypernatremia as described in previous papers.

This study counted the microorganisms isolated from bile and infection sites. More than three fourths (79.2%) of the patients had positive bile cultures, similar to previous studies (74.9%–88.4%) [4–6], but the microbial spectrum was not exactly the same. Klimczak et al. [46] found that *Streptococcus mitis* was the most common (43.8%) bacterial pathogen in the bile in tumor-related obstructive jaundice. In the present study, *S. mitis* was isolated from only two bile specimens, and those were from the same patient. It is well recognized that *S. mitis* is a normal component of the human oral commensal flora, so distinguishing the source of bacteria, including possible contamination or bacterial translocation, was difficult, especially in patients undergoing EBS for palliative treatment. We also noticed that only 3 of the 16 patients enrolled had tumor growing in the hepatic hilum, and *S. mitis* was cultured from just one of them, which might indicate that the microbial spectrum of PHCC differs from that of distal biliary obstruction. A study performed by Okamura et al. [47] concluded that pre-operative bile culture-targeted prophylactic antibiotics decreased SSIs. Taking into account the microbial spectrum, third-generation cephalosporins might be an excellent prophylactic antibiotic for patients without a positive bile culture before hepatectomy with cholangiojejunostomy for the treatment of PHCC.

With regard to the relation between PBD and post-operative infectious complications, our conclusion is in accord with that of Nagoya University [4], that is, PBD is unlikely to increase the incidence of infections ($p > 0.05$). Although PBD could lengthen pre-operative hospital stays (10 days vs. 32 days; $p < 0.05$), considering the positive role of PBD in improving surgical safety and long-term prognosis [3,4], it is recommended that appropriately selected patients undergo PBD.

The consistency between isolated microorganisms from bile and infection sites was 64.3%, lower than was found by Sugawara et al. (73.8%–88.9%) [4], the reason for which was that transanastomotic biliary stents were not used routinely in our patients, so monitoring post-operative bile culture routinely was impossible. We also noted that only 6 of 18 patients had microorganisms isolated from infectious sites in both pre-operative and intra-operative bile. Therefore, it is of great importance to monitor the pathogens regularly throughout the hospital course.

To the best of our knowledge, there are few studies focusing on early prediction of infectious complications after hepatectomy with cholangiojejunostomy for the treatment of PHCC. In this study, we investigated predictive factors and the microbial spectrum for infectious complications, proposed selections of prophylactic antibiotic, and emphasized the importance of pathogen surveillance. However, there are several limitations of this study. First, it is a retrospective study from a single center, which limits the extrapolation of conclusions to some extent. Differences in the surgical procedures and peri-operative management at different centers may contribute to bias of conclusions. It is necessary to design prospective studies to draw a more reliable conclusion. Second, in patients with infection by more than one microorganism, it is difficult to determine exactly which microorganism is responsible for the infection. Lastly, because of our small sample, we did not perform subgroup analysis for each infectious complication, the reasons for which may be different. Further studies are needed to validate our conclusions.

Being male, erythrocytopenia, hypocholesterolemia, and hypernatremia on POD 1 are independent risk factors for infectious complications, which might serve as monitoring indicators and therapeutic targets. According to our microbial spectrum, for patients without a positive bile culture, third-generation cephalosporins could be considered as the prophylactic antibiotic pre-operatively. Although bile culture can provide much valuable information, it still is necessary to monitor the pathogens throughout the hospital stay.

Author Disclosure Statement

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LM and YQ conceived the original idea, designed the study, supervised the execution, and helped revise the manuscript. XC, SS, and XY collected the data, performed the statistical analysis, and wrote the manuscript. XF, YF, and DC provided specialty support and helped revise and submit the manuscript. All authors thus contributed extensively to the work presented in this paper and have no personal conflicts of interest to declare.

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