

Supplementary material

Supplementary file 1. docx. Additional method.

Determination of acylcarnitines

The capillary voltage of positive ionization mode was maintained at 1.5 KV, cone voltage at 20 V, and desolvation temperature at 500 °C. The gas flow rate of desolvation was 1000 L/h, and the cone gas flow rate was 10 L/h. The chromatographic separation conditions were as follows: chromatographic column was Waters ACQUITY UPLC HSS T3 1.8 μm, 2.1×150 mm, mobile phase was A (water, 0.1% formic acid) and B (acetonitrile), flow rate was 0.3 mL/min, injection volume was 5.0 μL, column temperature was 50 °C. The gradient elution program was as follows: 0–2.0 min: 2% B; 2.0–5 min: linear gradient from 2 to 50% B; 5–11 min: linear gradient from 50 to 100% B; column re-equilibration from 11.5–13.0 min at 2% mobile phase B. The peak area of targeted metabolome data was integrated by the TargetLynx XS quantitative software, and the allowable error of retention time was 15s. The concentration was calculated *via* the single point isotope internal standard method.

Supplementary file 2. docx. Additional method.

Next-generation sequencing

Briefly, the extracted DNA was fragmented to yield 300bp fragments and further end-repaired, ligated to adapters and amplified using PCR with the PMseq™ high throughput gene detection kit for infectious pathogens (combined probe anchored polymerization sequencing method, BGI-Shenzhen, China, No. RM0438), according to the manufacturer's instructions. Based on the qualified double strand DNA library, single-stranded circular DNA library was then generated through DNA-denaturation and circularization. Then DNA nanoballs (DNBs) were formed by rolling circle amplification (RCA) using a universal kit for sequencing reaction (Combinatorial Probe-Anchor Synthesis,

BGI-Shenzhen, China, No. RM0170). DNBs were qualified by Qubit® ssDNA Assay Kit (Thermo Fisher Scientific) and were further sequenced by MGISEQ-2000 platform (MGI, China). Using bioinformatics analysis methods and pathogenic microorganism database, the types of pathogenic microorganisms obtained by sequencing were analyzed, and the detection results of each sample were obtained.

Supplementary Tables

Supplementary Table 1 Transitions for profiling acylcarnitines

Short name	Biochemical Name	Precursor (m/z)	Product Ion (m/z)	Cone (V)	Collision (V)	Rt (min)
C0	DL-Carnitine	162.2	85	20	18	1.13
C2	Acetyl-L-carnitine	204.1	85	20	18	1.35
C3	Propionyl-L-carnitine	218.1	85	20	18	1.45
C4	Butyryl-L-carnitine	232.2	85	20	18	4.98
C5	Valeryl-L-carnitine	246.2	85	20	18	5.23
C6	Hexanoyl-L-carnitine	260.2	85	20	18	5.67
C8	Octanoyl-L-carnitine	288.2	85	20	18	6.06
C8:1	Octenoyl-L-carnitine	286.2	85	20	18	5.87
C10	Decanoyl-L-carnitine	316.2	85	20	18	6.44
C10:1	Decenoyl-L-carnitine	314.2	85	20	18	6.26
C10-OH	Hydroxydecanoyl-L-carnitine	332.2	85	20	18	6.04
C12	Dodecanoyl-L-carnitine	344.3	85	20	18	6.92
C12:1	Dodecenoyl-L-carnitine	342.3	85	20	18	6.73
C12:0-OH	Hydroxydodecanoyl-L-carnitine	360.2	85	20	18	6.41
C12:1-OH	Hydroxydodecenoyl-L-carnitine	358.2	85	20	18	6.23
C12-DC	Dodecanedioyl-L-carnitine	374.2	85	20	18	7.45
C13	Tridecanoyl-L-carnitine	358.3	85	20	18	7.09
C14	Tetradecanoyl-L-carnitine	372.3	85	20	18	7.44
C14:1	Tetradecenoyl-L-carnitine	370.2	85	20	18	7.22
C14:2	Tetradecadienyl-L-carnitine	368.2	85	20	18	6.89
C14:0-OH	Hydroxytetradecanoyl-L-carnitine	388.2	85	20	18	6.89
C14:1-OH	Hydroxytetradecenoyl-L-carnitine	386.2	85	20	18	6.68
C14:2-OH	Hydroxytetradecadienyl-L-carnitine	384.2	85	20	18	6.46
C16	Hexadecanoyl-L-carnitine	400.3	85	20	18	7.95
C16:1	Hexadecenoyl-L-carnitine	398.3	85	20	18	7.62
C16:2	Hexadecadienyl-L-carnitine	396.2	85	20	18	7.31
C16-OH	Hydroxyhexadecanoyl-L-carnitine	416.2	85	20	18	7.45
C16:1-OH	Hydroxyhexadecenoyl-L-carnitine	414.2	85	20	18	7.11
C16:2-OH	Hydroxyhexadecadienyl-L-	412.2	85	20	18	6.82

OH	carnitine					
C17	Heptadecacyl-L-carnitine	414.4	85	20	18	8.20
C18	Octadecanoyl-L-carnitine	428.4	85	20	18	8.45
C18:1	Octadecenoyl-L-carnitine	426.4	85	20	18	8.08
C18:2	Octadecadienyl-L-carnitine	424.2	85	20	18	7.75
C18-OH	Hydroxyoctadecanoyl-L-carnitine	444.0	85	20	18	7.80
C18:1-OH	Hydroxyoctadecenoyl-L-carnitine	442.2	85	20	18	7.55
C18:2-OH	Hydroxyoctadecadienyl-L-carnitine	440.2	85	20	18	7.26
C20	Icosyl-L-carnitine	456.4	85	20	18	8.88
C20:4	Arachidonoyl-L-carnitine	448.0	85	20	18	7.75
C22	Behenoyl-L-carnitine	484.4	85	20	18	9.30

Supplementary Table 2 Comparison of clinical characteristics between patients with and without positive blood culture and blood mNGS results

Variables	Blood culture and mNGS results		P value
	Positive (n=92)	Negative (n=188)	
Demographic data			
Age (years)	74 (66,84)	74 (66,84)	0.903
Male (n %)	46 (50.0)	120 (63.8)	0.027
Comorbidities, n (%)			
CHD	21 (22.8)	57 (30.3)	0.189
Heart failure	8 (8.7)	15 (8.0)	0.837
Hypertension	21 (22.8)	66 (35.1)	0.037
CVD	14 (15.2)	19 (10.1)	0.213
COPD	5 (5.4)	5 (2.7)	0.200
Diabetes mellitus	20 (21.7)	44 (23.4)	0.755
Severity of cholangitis, n (%)			0.132
Mild	15 (16.3)	50 (26.6)	
Moderate	28 (30.4)	56 (29.8)	
Severe	49 (53.3)	82 (43.6)	
Biliary drainage, n (%)			0.016
No	25 (27.2)	79 (42.0)	
ERCP/PTCD	67 (72.8)	109 (58.0)	
Infection data			
Temperature (°C)	37.9 (37.1, 38.8)	37.3 (36.6, 38.2)	0.001
WBC count (×10 ⁹ /L)	12.51 (8.74, 16.78)	9.81 (7.00, 14.35)	0.001
Liver function			
TBIL (μmol/L)	101.75 (68.09, 139.39)	95.88 (59.53, 148.27)	0.834
DBIL (μmol/L)	70.29 (46.38, 95.40)	70.92 (38.70, 100.87)	0.920
ALT (U/L)	150.00 (87.10, 309.50)	147.50 (75.50, 271.00)	0.342
AST (U/L)	158.25 (92.50, 338.70)	137.30 (78.13, 271.48)	0.078
SOFA score	3 (1.25, 4.75)	2 (1, 3)	0.004

Data expressed as median (P₂₅, P₇₅) or n (%). mNGS: Metagenomic next generation sequencing; CHD: Coronary heart disease; CVD: Cerebral vascular disease; COPD: Chronic obstructive pulmonary disease; ERCP: Endoscopic retrograde cholangiopancreatography; PTCD: Percutaneous transhepatic biliary drainage; WBC: White blood cells; TBIL: Total bilirubin; DBIL: Direct bilirubin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; SOFA: Sequential organ failure assessment.

Supplementary Table 3 Diagnostic value of presepsin, procalcitonin, and acylcarnitines for bloodstream infection in acute cholangitis

Variables	AUC (95% CI)	<i>P</i> value	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
Presepsin (pg/mL)	0.610 (0.532-0.688)	0.011	1147.5	91.3	31.9	57.3	78.6	1.34	0.27
Procalcitonin (ng/mL)	0.679 (0.605-0.753)	<0.001	10.83	71.7	58.0	63.1	67.2	1.71	0.49
C2 (μmol/L)	0.599 (0.515-0.682)	0.023	14.59	37	84	69.8	57.1	2.31	0.75
C12:1 OH (μmol/L)	0.603 (0.517-0.689)	0.018	0.0242	48.4	76.4	67.2	59.7	2.05	0.68
Temperature (°C)	0.639 (0.558-0.719)	0.001	37.1	75	49.3	59.7	66.4	1.48	0.51

C2: Acetyl-L-carnitine; C12:1 OH: Hydroxydodecenoyl-L-carnitine; AUC: Area under the receiver operating characteristic curve; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value; LR+: Positive likelihood ratio; LR-: Negative likelihood ratio.

Supplementary Table 4 Comparison of clinical characteristics between survival and non-survival groups

Variables	28-day survival outcome		<i>P</i> value
	Survival (n=271)	Non-survival (n=9)	
Demographic data			
Age, years	74 (66,84)	83 (71,89)	0.153
Male	161 (59.4)	5 (55.6)	0.536
Comorbidities, n (%)			
CHD	74 (27.3)	4 (44.4)	0.220
Heart failure	20 (7.4)	3 (33.3)	0.029
Hypertension	82 (30.3)	5 (55.6)	0.109
CVD	33 (12.2)	0 (0.0)	0.318
COPD	7 (2.6)	0 (33.3)	0.002
Diabetes mellitus	58 (21.4)	6 (66.7)	0.005
Severity of cholangitis, n (%)			0.005
Mild	65 (24.0)	0 (0.0)	
Moderate	84 (31.0)	0 (0.0)	
Severe	122 (45.0)	9 (100.0)	
Biliary drainage, n (%)			0.555
No	101 (37.3)	3 (33.3)	
ERCP/PTCD	170 (62.7)	6 (66.7)	
Infection data			
Temperature (°C)	37.5 (36.7, 38.5)	36.7 (36.0, 38.6)	0.135
WBC count (×10 ⁹ /L)	10.72 (7.36, 14.78)	15.71 (7.44, 21.65)	0.229
Liver function			
TBIL (μmol/L)	98.68 (62.24, 140.52)	118.60 (76.03, 163.27)	0.481
DBIL (μmol/L)	70.13 (42.11, 98.81)	72.07 (56.19, 108.02)	0.456
ALT (U/L)	148.00 (81.00, 288.00)	147.00 (51.00, 416.50)	0.862
AST (U/L)	138.00 (81.25, 283.05)	278.60 (93.05, 799.00)	0.176
SOFA score	2 (1,4)	8 (5,11)	0.000

Data expressed as median (P₂₅, P₇₅) or n (%). CHD: Coronary heart disease; CVD: Cerebral vascular disease; COPD: Chronic obstructive pulmonary disease; ERCP: Endoscopic retrograde cholangiopancreatography; PTCD: Percutaneous transhepatic biliary drainage; WBC: White blood cells; TBIL: Total bilirubin; DBIL: Direct bilirubin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; SOFA: Sequential organ failure assessment.

Supplementary Table 5 Predictive value of presepsin, acylcarnitines, and SOFA score for 28-day mortality in acute cholangitis.

Variables	AUC (95% CI)	<i>P</i> value	Cut- off	Sensitivity (%)	Specificity (%)	PPV	NPV	LR+	LR-
Presepsin (pg/mL)	0.839 (0.734-0.945)	0.002	1850.5	100	51.3	67.2	100	2.05	0.00
SOFA	0.848 (0.611-1.000)	0.002	5.5	85.7	93.0	92.4	86.7	12.24	0.15
C2 (μ mol/L)	0.880 (0.728-1.000)	0.001	17.07	85.7	87.9	87.6	86.0	7.08	0.16
C12:1 OH (μ mol/L)	0.822 (0.698-0.946)	0.004	0.019	100	60.8	71.8	100	2.55	0.00

SOFA: Sequential organ failure assessment; C2: Acetyl-L-carnitine; C12:1 OH: Hydroxydodecenoyl-L-carnitine; AUC: Area under the receiver operating characteristic curve; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value; LR+: Positive likelihood ratio; LR-: Negative likelihood ratio.

Supplementary Table 6 Variables associated with 28-day mortality (multivariate Cox proportional hazards models)

Variables	B	SE	Wald	df	HR	95% CI	P value
C2	0.074	0.026	8.133	1	1.077	1.023–1.133	0.004
Presepsin	0.000	0.000	0.117	1	1.000	0.999-1.000	0.732
C12: 1 OH	-1.368	10.752	0.016	1	0.255	0.000-3.614*10 ⁸	0.899
Severity grading	9.297	96.456	0.009	1	1.09*10 ⁴	0.000-1.384*10 ⁸⁶	0.923
SOFA score	0.165	0.110	2.261	1	1.180	0.951-1.464	0.133

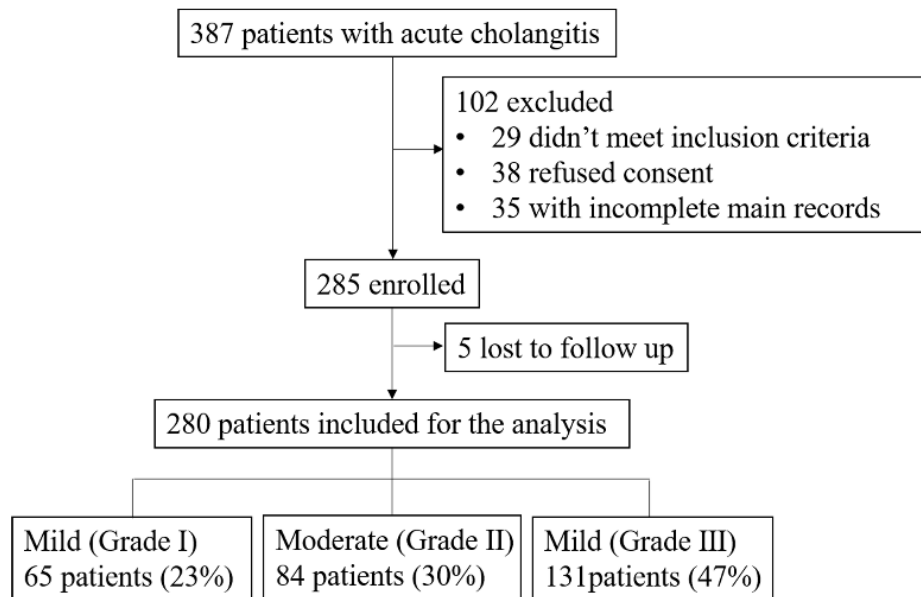
C2: Acetyl-L-carnitine; C12:1 OH: Hydroxydodecenoyl-L-carnitine; SE: Standard error; df: Degrees of freedom; HR: Hazard ratio; CI: Confidence interval.

Supplementary Table 7 Correlation between presepsin or procalcitonin and C2

Variables	Presepsin	Procalcitonin	C2	C4
SOFA	$r = 0.424$ $P < 0.001$	$r = 0.357$ $P < 0.001$	$r = 0.208$ $P < 0.001$	$r = 0.193$ $P = 0.001$
TBIL	$r = 0.290$ $P < 0.001$	$r = -0.109$ $P = 0.069$	$r = 0.059$ $P = 0.324$	$r = -0.041$ $P = 0.499$
DBIL	$r = 0.304$ $P < 0.001$	$r = -0.057$ $P = 0.345$	$r = 0.068$ $P = 0.255$	$r = -0.042$ $P = 0.486$
ALT	$R = -0.007$ $P = 0.912$	$r = 0.114$ $P = 0.057$	$r = -0.138$ $P = 0.021$	$r = -0.092$ $P = 0.126$
AST	$r = 0.015$ $P = 0.806$	$r = 0.151$ $P = 0.012$	$r = -0.119$ $P = 0.049$	$r = -0.029$ $P = 0.636$
Presepsin	-	$r = 0.431$ $P < 0.001$	$r = 0.270$ $P < 0.001$	$r = 0.187$ $P = 0.002$
Procalcitonin	$r = 0.431$ $P < 0.001$	-	$r = 0.094$ $P = 0.118$	$r = 0.172$ $P = 0.004$

C2: Acetyl-L-carnitine; SOFA: Sequential organ failure assessment; TBIL: Total bilirubin; DBIL: Direct bilirubin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Supplementary Figures



Supplementary Figure 1 Study flowchart.