Table 1: Demographics of study population

Characteristic	**Tumour site**				**Healthy**
	All cases	**iCCA**	**pCCA**	**dCCA**	
Participants, n	52	23	21	8	46
Age, mean, (range), y	59.0 (35–76)	59.7 (38–68)	**59.0 (37–76)**	55.4 (35–72)	31.1 (22-62)
Male, %	53.7	47.8	**57.1**	62.5	26.1
Serum biochemistry**,** mean, (range),					
Alanine aminotransferase, (U/L)	57.4 (7 – 529)	30.2 (7 – 105)	67.1 (14 –163)	119.4 (20 –529)	-
Aspartate aminotransferase, (U/L)	73.6 (13 – >700)	37.8 (13 – 110)	82.5 (23 –311)	164.9 (32 –700)	-
Alkaline phosphatase, (U/L)	324 (49 – 1124)	144.6 (49 – 367)	295.6 (74–1124)	321.2 (63 – 843)	-
Biliary obstruction (jaundice %)	46.1	0	85.7	75	0

.

Table 2: Differential metabolites between cholangiocarcinoma patients and controls.

m/z	RT	Compound	Class	Adduct	Mass Error	VIP
396.044	3.86	Ceftriaxone	Antibiotic	Fragment	1	6.2
172.072	2.89	Metronidazole	Antibiotic	M+H	1	6.1
555.054	3.86	Ceftriaxone	Antibiotic	M+H	1	6.1
128.045	2.89	Metronidazole	Antibiotic	Fragment	1	5.0
105.033	3.80	Hippurate	Gut microbiota metabolite	Fragment	2	4.4
362.151	3.98	Ofloxacin	Antibiotic	M+H	0	4.0
459.274	4.20	Prednisolone tebutate	Steroid	M+H	0	4.0
152.071	2.42	Paracetamol	Analgesic	M+H	2	3.9
289.227	5.01	Bupivacaine	Local anaesthetic	M+H	1	3.7
503.306	4.31	Unknown Drug				3.7

Table 3: The metabolic profile altered in cholangiocarcinoma patients with obstructive jaundice.

m/z	RT	Metabolite	Adduct	Trend in jaundice	VIP	p value	FC	Identity*
114.066	0.51	Creatinine	M+H	D	2.5	0.851	-1.05	a
132.077	0.53	Creatine	M+H	D	2.4	0.659	-1.51	b
144.102	0.58	Proline betaine	M+H	D	2.6	0.577	-1.30	b
191.018	0.91	Isocitrate	M-H	D	4.4	0.034	-1.60	b
191.017	1.02	Citrate	M-H	D	1.8	0.680	-1.30	a
232.154	2.80	Butyryl-L-carnitine (C4)	M+H	D	2.3	0.271	-1.25	a
211.058	3.29	Vanilpyruvate	M+H	I	3.5	<0.0001	2.56	b
318.191	3.78	Acylcarnitine (C9-OH)	M+H	I	4.6	0.019	1.97	c
178.05	3.80	Hippurate	M-H	D	2.8	0.808	-1.23	a
212.001	3.81	Indoxylsulfate	M-H	D	10	0.437	-1.24	a
243.032	5.31	4-phenylbutanic acid-O-sulfate	M-H	D	2.8	0.424	-10.7	b
514.284	5.91	Taurocholic acid	M-H	I	4.1	0.008	75.0	b
544.258	5.91	Glycocholic acid sulfate	M-H	I	3.5	<0.0001	7.66	b
328.248	6.13	Acylcarnitine (C10:2-OH)	M+H	I	3.6	0.478	1.42	c
624.338	6.14	Glycochenodeoxycholic acid 3-glucuronide	M-H	I	2.8	<0.0001	12.5	b
464.299	6.23	Glycocholic acid	M-H	I	2.9	<0.0001	4.07	a
466.316	6.24	Glycocholic acid	M+H	I	3.8	<0.0001	4.52	a
528.262	6.33	Glycochenodeoxycholate-N-sulfate	M-H	I	7.4	0.0002	10.4	b
528.262	6.50	Glycochenodeoxycholate-N-sulfate	M-H	I	7.1	<0.0001	4.72	b

FC= fold change; RT= retention time; VIP= variable importance in projection score.

*Level of metabolite identification: (a) Identified compound; (b) putatively annotated compound; and (c) putatively characterised compound class.

D = decreased; I = increased

Table 4: The metabolic profile altered in non-jaundiced cholangiocarcinoma patients compared to controls.

m/z	RT	Metabolite	Adduct	Trend in CCA	VIP	p value	FC	Identification*
144.102	0.5	Proline betaine	M+H	I	5.9	0.006	2.74	b
96.958	0.6	Sulfate	M-H	I	3.0	0.996	1.02	b
191.018	0.9	Isocitrate	M-H	D	3.3	<0.0001	-1.29	b
229.154	0.9	Leucyl-proline	M+H	I	3.7	0.043	1.45	b
191.017	1.0	Citrate	M-H	D	3.0	0.294	-1.36	a
166.072	1.1	7-Methylguanine	M+H	I	2.7	0.040	1.30	b
120.08	2.4	Phenylalanine†	+	D	4.3	0.878	-0.92	b
188.985	3.0	Pyrocatechol sulfate	M-H	D	4.1	0.014	-2.06	b
318.191	3.7	Acylcarnitne (C9-OH)	M+H	I	2.3	0.166	2.24	c
105.033	3.8	Hippurate †	+	D	6.1	0.0003	-1.87	a
178.05	3.8	Hippurate	M-H	D	7.8	0.001	-1.96	a
212.001	3.8	Indoxylsulfate	M-H	I	8.4	0.843	1.38	a
263.102	3.8	Phenylacetylglutamine	M-H	I	2.9	0.213	1.25	b
332.207	4.1	Acylcarnitne (C10-OH)	M+H	I	2.5	0.004	3.13	c
293.147	4.9	Unknown	M+H	D	3.0	<0.0001	-1.91	b
286.201	4.9	Acylcarnitne (C8:1)	M+H	D	5.7	<0.0001	-2.91	c

310.201	5.2	Acylcarnitne (C10:3)	M+H	D	5.4	<0.0001	-3.45	c
300.217	5.2	UnidentĮed acylcarniϴne	M+H	D	3.3	0.162	-1.49	c
539.248	5.2	Tetrahydroaldosterone-3-glucuronide	M-H	D	2.0	<0.0001	-1.67	b
243.032	5.3	4-phenylbutanic acid-O-sulfate	M-H	I	4.4	0.350	7.31	b
383.152	5.3	3b,16a-Dihydroxyandrostenone sulfate	M-H	D	2.7	<0.0001	-4.03	b
302.233	5.5	2,6 dimethylheptanoyl carniϴne (C9:0)	M+H	D	3.0	0.013	-1.77	b
312.217	5.6	2-trans,4-cis-decadienoylcarniϴne (C10:2)	M+H	D	2.6	<0.0001	-3.75	b
314.233	5.8	Acylcarnitne (C10:1)	M+H	D	3.9	<0.0001	-2.02	c
331.174	5.9	17ɲ-hydroxyprogesterone	M-H	D	3.1	0.066	-1.54	b
367.157	6.3	Steroid sulfate (C19 H28 O5 S)	M-H	D	2.6	0.041	-3.53	c
465.248	6.3	Steroid glucuronide (C25 H38 O8)	M-H	D	2.3	<0.0001	-1.97	c
528.262	6.5	Glycochenodeoxycholate-N-sulfate	M-H	I	3.1	0.297	2.82	d
495.295	6.58	Pregnanediol-3-glucuronide	M-H	D	2.2	0.002	-4.20	b

FC= fold change; RT= retention time; VIP= variable importance in projection score.

*Level of metabolite identification: (a) Identified compound; (b) putatively annotated compound; (c) putatively characterised compound class; and (d) Unknown. † m/z represents a fragment of this metabolite. D = decreased; I = increased

Table 5: The areas under the curves (AUCs) of receiver operating characteristic (ROC) curves of individual markers.

Metabolite	Trend in CCA	AUC (%)	95% CI	Cut-off	Sensitivity (%)	Specificity (%)
Acylcarnitne:						
2-trans,4-cis-decadienoylcarniϴne	D	95.2	89.2 –	42	92.9	91.3
Acylcarnitine, (C10:3)	D	94.4	88.6 –	116	85.7	91.3
Acylcarnitine, (C8:1)	D	85.9	76.3 –	224	78.6	80.4
Acylcarnitine, (C10-OH)	I	84.4	75.2 –	61	75.0	78.3
Acylcarnitine, (C10:1)	D	83.1	72.0 –	188	71.4	84.8
2,6 dimethylheptanoyl carnitine,(C9:0)	D	74.0	62.1 –	172	60.7	89.1
Acylcarnitine, (C9-OH)	I	68.6	56.2 –	82	64.3	65.2
Unidentified acylcarnitine, 300.217	D	70.9	56.4 –	265	76.1	67.9
***Steroids**:*						
3b,16a-Dihydroxyandrostenone sulfate	D	88.4	79.9 –	77	84.8	85.7
Steroid glucuronide (C25 H38 O8)	D	86.4	75.8 –	181	84.9	85.7
Tetrahydroaldosterone-3-glucuronide	D	83.5	72.9 –	139	80.4	78.6
Pregnanediol-3-glucuronide	D	78.9	67.5 –	41	60.9	85.7
Steroid sulfate (C19 H28 O5 S)	D	78.4	65.8 –	49	69.6	75.0
17ɲ-hydroxyprogesterone	D	63.5	51.1 –	523	67.9	56.5
Other:						
Glycochenodeoxycholate-N-sulfate	I	85.5	75.1 –	46	78.6	82.6

Hippurate	D	74.7	62.6 –	1880	71.4	67.4
7-Methylguanine	I	72.9	59.0 –	505	64.3	76.1
Pyrocatechol sulfate	D	70.8	58.3 –	315	53.6	78.3
Leucyl-Proline	I	70.1	57.0 –	629	63.0	78.6
Isocitrate	D	65.1	50.7 –	1150	71.4	58.7
4-phenylbutanic acid-O-sulfate	I	62.8	48.4 –	7	50.0	80.4
Phenylacetylglutamine	I	61.6	47.5 –	1900	50.0	80.3
Proline betaine	I	61.8	45.4 –	539	53.6	89.1
Phenylalanine	D	60.3	45.6 –	439	57.1	73.9
Indoxylsulfate	I	60.1	44.6 –	3570	57.1	58.7
Sulfate	I	52.6	31.8 –	381	50.0	58.7
Citrate	D	52.4	39.1 –	481	64.3	58.7

AUC= area under the receiver operating characteristic curve; CI= confidence intervals; CCA= cholangiocarcinoma.

D = decreased; I = increased