

ONLINE RESOURCE 1

Metabolomic analysis of human cirrhosis and hepatocellular carcinoma: a pilot study

Digestive Diseases and Sciences

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Supplementary Table 1 Analysis of metabolomic data in HCC and LC control patients

	log2 fold-change (LC control vs. HCC)	mean conc. [μmol/L]	t-statistics	p-value	adjusted p-value
Octadecenoic acid	1.8	1369.9	10.29	< 0.0001*	< 0.0001
Biliverdin	1.3	2.1	3.27	0.00166*	0.03148
Pantothenic acid	1.0	0.3	3.14	0.00249*	0.03935
1-Methylhistidine	0.9	6.8	2.66	0.00966*	0.07151
Campesterol	0.9	13.9	2.55	0.01288*	0.08924
Pseudouridine	0.8	2.2	3.47	0.00091*	0.02836
N-Acetylcytidine	0.7	2.2	2.74	0.00789*	0.06875
N,N'-Dimethylarginine	0.7	0.7	2.94	0.00448*	0.05528
Sedoheptulose-7-phosphate	0.6	1.5	3.91	0.00021*	0.01006
trans-4-Hydroxyproline	0.6	15.1	2.78	0.00707*	0.06875
Thymine	0.6	1.5	2.72	0.00825*	0.06875
7-Methylguanosine	0.5	2.8	3.32	0.00144*	0.03144
Octadecadienoic acid	0.5	84.1	3.59	0.00061*	0.02167
2-Hydroxybutyrate	0.4	67.0	2.59	0.00381*	0.05414
Trihexosylceramide	0.4	1.6	3	0.01165*	0.08271
O-Acetylcarnitine	0.4	11.4	2.75	0.00753*	0.06875
Citrate	0.4	115.5	2.89	0.00515*	0.05853
3-Hydroxyisobutyrate	0.4	24.6	2.94	0.00444*	0.05528
Asparagine	0.3	53.2	2.98	0.0011*	0.02836
Lactosylceramide	0.3	0.8	3.41	0.00401*	0.05422
Phenylalanine	0.3	86.0	2.67	0.00938*	0.07151
Choline ether lipid	0.3	5.1	3.65	0.00012*	0.00691
Plasmenyl choline	0.3	19.4	4.08	0.00051*	0.02085
Plasmanyl choline	0.2	1.5	2.71	0.0084*	0.06875
Lysophosphatidylcholine	-0.3	8.6	-3.29	0.00159*	0.03148
Diacylglycerol	-0.3	1.8	-2.75	0.00753*	0.06875
Adenine	-0.3	1.6	-2.72	0.00831*	0.06875
Lysophosphatidylethanolamine	-0.4	0.2	-2.9	0.00277*	0.04143
Phosphatidylethanolamine	-0.4	2.3	-3.1	0.00505*	0.05853
Eicosanoic acid	-0.4	15.3	-2.73	0.00751*	0.06875
Phosphatidylethanolamines,total	-0.4	122.9	-2.76	0.00804*	0.06875
Cystathionine	-0.6	3.4	-3.36	0.00127*	0.03008
Guanine	-0.6	1.8	-2.71	0.00847*	0.06875
Ergothioneine	-0.7	1.7	-2.68	0.0092*	0.07151
Docosahexaenoic acid	-0.8	130.0	-3.43	0.00103*	0.02836
1,5-Anhydrosorbitol	-0.8	73.2	-4.17	0.00009*	0.00691
Hippuric acid	-0.9	1.5	-2.66	0.00982*	0.07151
Cholesterylester C20:5	-0.9	47.6	-3.17	0.00225*	0.03762
2-Phosphoglycerate	-1.1	2.1	-4.09	0.00012*	0.00691
Glutamate	-1.2	82.9	-5.34	< 0.0001*	0.00016
Furoylglycine	-2.0	0.3	-3.23	0.00192*	0.03404

Positive values for log2 fold-change indicate a higher metabolite concentration in HCC patients vs. the LC-control group. Abbreviations: HCC: Hepatocellular carcinoma; LC: Liver cirrhosis. * shows a statistical significance (p<0.05).

Supplementary Table 2 Analysis of metabolomic data in HCC and LC control patients with alcoholic liver disease (n=13 vs. n=16)

	log2 fold-change (LC control vs. HCC)	FDR	p-value
7-Methylguanosine	-1.679830616	0.004105201	0.000200254*
3-Hydroxyisobutyrate	-11.99042235	0.004105201	0.000200254*
Octadecenoic acid	-1475.797804	0.007926854	0.000580014*
Thymine	-1.00615858	0.010243005	0.001149489*
2-Hydroxybutyrate	-32.43525704	0.010243005	0.001249147*
1,5-Anhydrosorbitol	40.69213338	0.020558369	0.003509965*
N-Acetylcytidine	-1.487077277	0.020558369	0.003509965*
Sedoheptulose-7-phosphate	-0.704200244	0.022301702	0.005725995*
Pseudouridine	-1.76072625	0.022301702	0.005616269*
Lysophosphatidylcholine	1.621318851	0.022301702	0.004817052*
N,N'-Dimethylarginine	-0.430782794	0.022301702	0.006527328*
O-Acetylcarnitine	-5.188972386	0.022301702	0.006527328*
Lysophosphatidylethanolamine	0.068367182	0.027559103	0.008738252*
trans-4-hydroxyproline	-8.243198435	0.029480633	0.010066557*
Citrate	-46.57803389	0.029631228	0.011563406*
Diacylglycerol	0.425864783	0.029631228	0.011563406*
Ergothioneine	0.754969908	0.036498279	0.015133433*
Biliverdin	-1.465963007	0.039276948	0.017243538*
1-Methylhistidine	-6.541928543	0.043338981	0.020083918*
Plasmenyl choline	-2.937269999	0.051489549	0.025116853*
Glutamate	38.30733218	0.088994586	0.049923792*
Cystathionine	1.528428677	0.088994586	0.045982625*
Pantothenic acid	-0.309369142	0.088994586	0.049923792*
Furoylglycine	0.545460253	0.105305349	0.061642155
Phenylalanine	-23.12664428	0.111970594	0.068274752
Choline ether lipid	-0.755522939	0.131247502	0.083230123
Asparagine	-12.07262221	0.200170635	0.131819687
2-Phosphoglycerate	1.10356976	0.203131766	0.141224047
Plasmanyl choline	-0.270253515	0.203131766	0.143678566
Trihexosylceramide	-0.472736597	0.247368513	0.181001351
Lactosylceramide	-0.079540374	0.329942111	0.249468425
Eicosanoic acid	3.529831227	0.368363726	0.287503396
Cholesteryl ester C20:5	12.17301405	0.371255044	0.307870037
Phosphatidylethanolamines, Total	11.65770534	0.371255044	0.307870037
Octadecadienoic acid	-23.69488449	0.411524629	0.351301513
Adenine	0.247324195	0.446524	0.392069853
Campesterol	-2.688583141	0.826726101	0.746069896
Docosahexaenoic acid	9.967060253	0.84050897	0.779008314
Guanine	-0.128292084	0.95323091	0.929981376
Hippuric acid	0.277423185	0.95323091	0.914083073
Phosphatidylethanolamine	0.032515686	1	1

Positive values for log2 fold-change indicate a higher metabolite concentration in HCC patients vs. the LC-control group. Abbreviations: HCC: Hepatocellular carcinoma; LC: Liver cirrhosis. * shows a statistical significance (p<0.05).

Supplementary Table 3 Analysis of metabolomic data in HCC with local ablation vs. palliative care

	log2 fold-change (palliative vs. local ablation)	mean conc. [μmol/L]	t-statistics	p-value	adjusted p-value	log2 fold-change (LC control vs. HCC.)
Octadecenoic acid	1.24	599.5	2.97	0.00373*	0.00466*	1.24
Octadecadienoic acid	0.18	70.1	3	0.0034*	0.00466*	0.18
Lysophosphatidylcholine	-0.52	3.0	-3.21	0.00176*	0.00441*	-0.52
Campesterol	-0.71	9.3	-3.54	0.00059*	0.00297*	-0.71
Furoylglycine	-1.08	0.7	-2.84	0.00538*	0.00538*	-1.08

Positive values for log2 fold-change indicate higher metabolite concentrations in HCC patients from the palliative treatment group vs. local ablation group. Abbreviations: HCC: Hepatocellular carcinoma; LC: Liver cirrhosis. * shows a statistical significance (p<0.05).

**Suppl. Table 4 Multivariate analysis regarding association between OS and metabolites
in HCC patients**

	HR	95 % CI	Wald-p	Log-rank-p
Octadecenoic acid	3.66	1.31-10.24	0.0135*	0.02207*
Isocitrate	2.56	0.8-8.17	0.11164	0.11539
Pseudouridine	2.00	0.74-5.43	0.17469	0.12651
2-Hydroxybutyrate	2.29	0.9-5.85	0.08268	0.05426
Erythrol	2.40	0.78-7.42	0.12697	0.10014
Erythronic acid	1.69	0.59-4.88	0.33184	0.16969
Myo-inositol	1.02	0.44-2.35	0.95876	0.24934
Creatinine	3.37	1.24-9.17	0.0171*	0.02724*

Stated are the hazard ratios (HR) with the 95% CI for the eight metabolites showing association with OS in HCC patients adjusted for possible confounding factors. Variables with p<0.200 in univariate analysis were included in the model: CPS, BCLC stage and treatment type. Abbreviation: BCLC: Barcelona Clinic Liver Cancer; CI: Confidence interval; CPS: Child-Pugh score; HCC: Hepatocellular carcinoma. * shows a statistical significance (p<0.05).