# Longitudinal metabolomics investigations of blood, urine and fecal matrices for the stratification of cirrhotic patients with acute-on-chronic liver failure (ACLF)



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Sebastian D. Burz<sup>1</sup>, Sylvain Dechaumet<sup>1</sup>, Emeline Chu-Van<sup>1</sup>, Florence Castelli<sup>1</sup>, Eric Venot<sup>1</sup>, Etienne Thevenot<sup>1</sup>, François Fenaille<sup>1</sup>, Jonel Trebicka<sup>2,3</sup>, Christophe Junot<sup>1</sup>

1 Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), SPI, MetaboHUB, Gif-Sur-Yvette, France 2 Translational Hepatology, Departement of Internal Medicine I, Goethe University Clinic, Frankfurt, Germany

**3** European Foundation for the Study of Chronic Liver Failure, Barcelona, Spain

**Contact: burzsd@gmail.com** 

## ABSTRACT

Acute decompensation (AD) and its progression to acute-onchronic liver failure (ACLF) are associated with intense systemic inflammation, multiple organ dysfunctions, and a major risk of short-term mortality. Within the 22 European institutions joining forces in the MICROB-PREDICT project to improve the prevention and treatment of cirrhosis, we aim to find new prognostic biomarkers of ACLF development by using untargeted metabolomics.





#### Fig. 2 Metabolic signature enabling to discriminate between ACLF (yes /no)



	nd= not d	etected			G1.G2.G3.G	4 G1.G2.G	3.G4 G10	52.G3.G4	IG1/G3	GNG3	G1/G3	G1/G3	G1/G3	G1/G3
- 1	p-value = p-value BH				Feces	Sera	Urin	ve	Feces	Feces	Sera	Sera	Urine	Urine
- 1	annot_spi_metab id stat			id status	p-value	p-value	е p-v	alue	p-value	ratio	p-value	ratio	p-valu	e ratio
	2-hydroxycaproic acid		0,006	0,000	0.00	10	0.439	1,774	0.000	1,650	0.007	0.646		
- 1	(MHPG) 4-Hydroxy-3-													
	methoxyphenylglycol sulfate a.c.d			0,004	0,000	0,00	0/0,025	0.023	8.546	0.000	3.038	0.000/0.	795 1,905/0,989	
	Caffeine Homovanillic-acidIsohomova a.c Guanidinosuccinic acid a.c.d Hydroxykynurenine			0,024	0,029	0,01	7	0.120	2.004	0.275	1.721	0.278	1.512	
- 1			a,c	0,016/0,017	0,000	0,00	00,0010,002	1.000/0.109	0.662/1830	0.000	1.878	0.000/0	007 2 826/1 949	
1			a.c.d	0,000 0,000		0,00	0	0.003	12.565	0.000	11.133	0.000	2.427	
1			0,000 0,000		0,000		0.068 0.578	0.000	2,890	0.961	1260			
	Kunurenia	c-acid		a.b.d	0.032	0.000	0,00	0	0.566	1.441	0.000	2 729	0.000	1.915
1	L-Kynurenine N-acetyl-DL-tryptophan a.c.d Tryptophan/DL-TryptophanL-Tryptopha 5-hudrowtruptophan a.b				0.001	0.000	0.03	15	0.000	1700	0.000	1.472	1,000	1,002
- 1			an i	a.c.d	0.000	0.001	0.00	0	0.020	2 201	0.000	2 660	0.000	2,500
- 1			Truptophar	0.013/0.046	0.006	0.02	0/0.035	0,030	1,201	0,001	2,003	0,000	3,032	
- 1			ab	0.006/0.031	0.000	0.00	12	0,488/0,566	1,0/0/1,066	0,043	0,781	0,0220,	0,653(0,68	
- 1	N-åretul-l	-phepulala	mine	abd	0.003	0.000	0.00		0,336/0,735	1,506/1,220	0,000	2,363	0,135	1,232
	Deteroute	amitine		abd	0.017	0.000	0.04	1	0,120	1,682	0,000	1,593	0,000/10	1,74/1,106
	Imidazola	lactic acid		4,0,0	0.022	0.002	0.01	2	0,094	3,920	0,005	1,420	0,186	1,622
	Adapitall	-(-)-Arabito	UD-(+)-Ar	abital	0.0120.017	0.000	0.02	9	0,205	1,821	0,001	1,365	0,016	0,658
	N-Acetal.	etul-1-methiopipeth-Acetul-D-penici		dullui I-D-panicil	0.0120.041	0.0000	0,02		0,209/0,220	2,204/2,384	0,000	1,931	0,096	1,257
	генсекун	L-merioriir	ienv-Aces	n-p-perior	0,0120,041	0,00000,0	00 0,00	14	0,889/1,000	1,4010,460	0,000/0,00	0 2,026/1,6	26 0,850	1,208
ected			G2/G3	G2/G3	G2/G3	G2/G3	G2/G3	G2/G3	G3/G4	G3/G4	G3/G4	G3/G4	G3/64	G3/G4
value B	H		Feces	Feces	Sera	Sera	Urine	Urine	Feces	Feces	Sera	Sera	Urine	Urine
metab	)	id status	p-value	ratio	p-value	ratio	p-value	ratio	p-value	ratio	p-value	ratio	p-value	ratio
proic a	cid		0,342	2,240	0,730	1,113	0,066	0,632	0,062	0,240	0,170	0,736	0,048	2,091
ydroxy-	-3-													
nyigiye	ol sulfate	a,c,d	1,000	1,779	0,064	1,614	0,060/0,37	7 1,623/1,374	0,349	0,151	0,019	0,566	1,000/1,000	1,196/1,069
			1,000	1,112	1,000	0,792	0,995	0,741	1,000	1,391	0,635	1,567	0,809	1,599
-acidil:	sohomova	a,c	0,387/1.00	0 0,462/1,304	1,000	1,118	0,563/1,000	1,623/0,933	0,0690,492	2,3250,495	0,266	0,739	0,660/1000/	0,4790,9810,89
accinic	acid	a,c,d	0,192	7,628	0,007	4,428	0,021	1,819	0,154	0,145	0,000	0,044	0,002	0,327
urenine	,		0,025	0,332	0,002	2,024	0,011	3,059	0,122	2,304	0,278	0,803	0,915	0,543
cid		a,b,d	1,000	1,008	0,097	1,474	0,087	1,425	0,105	0,373	0,000	0,360	0,809	0,753
e			0,073	1,700	0,024	1,278	0,140	1,909	0,188	0,586	0,020	0,657	1,000	0,613
tryptop	han	a,c,d	0,052	2,306	1,000	1,473	0,675	2,145	0,080	0,375	0,677	0,564	0,639	0,353
DL-Try	ptophanL	Tryptophar	0,093/0,27	7 2,289/2,015	0,091	0,689	0,864/0,97	6 0,854/0,864	0,644/0,599	0,4720,545	0,863	1,178	1,000/1,000	1,206/1,193
ptopha	n	a,b	0,166/0,72	0 2,059/1,258	3 0,002	1,624	0,017	1,482	0,1490,088	0,414/0,573	0,000	0,449	1,000	1,032
henyla	lanine	a,b,d	0,522	1,599	1,000	1,147	1,000/0,570	1,229/1,535/	0,067	0,406	0,536	0,694	1,000/0,768/	0,819/1,621/0,97
nitine		a,b,d	0,849	2,189	1,000	1,041	0,476	1,625	0,449	0,151	0.034	0,604	0,901	0,597
tic acid	1		1,000	1,511	1,000	1,094	0,833	0,831	0,214	0,334	0,973	0,843	0,928	1,474
)-Arabi	Arabitol/D-(+)-Arabitol		0,192/0,144	4 2,702/6,25	3 0,000	1,569	0,397	1,255	0.744/1.000	0,420/0,642	0,001	0,552	1,000	1,112
48.7	in all the state	ul-D-papioil	0.300/0.07	1.6810 329	0.002/0.001	1.820/1.712	0.005	1,719	0,069/1,000	0,409/2,098	0.3410.281	0,6750,920	1.000	0,747

150 100 50 otal annotated molecules

G4: ACLF at admission

4 groups have been clinically defined at 3 months follow up: G1: ACLF development during the follow up G2: Unstable during follow up (died/readmission/no ACLF) G3: Stable during follow up(no died/no readmission/no ACLF)

There is a metabolomic signature between the 4 groups shared or specific of the 3 tissues



Fecal samples

Fig. 1 Sampling hepatic decompensation (1)

### **METHODS**

The untargeted metabolomic analysis of fecal, urine and serum samples (309 of each) collected from 94 patients over a 3months follow-up period was performed using two complementary liquid chromatography coupled to high-resolution mass spectrometry methods (2). Datasets were pre-processed on the W4M platform (3) and annotated with in-house spectral databases to obtain sets of confidently annotated metabolites: 220 in sera, 352 in urines, 388 in feces, with limited overlaps between matrices.

### RESULTS

Univariate and multivariate statistical analyses led to molecular signatures documenting the severity of decompensation and including 107; 136 and 113 metabolites in serum, urine and stool samples, respectively.

#### Fig. 3 Metabolic signature enabling to discriminate between G1,G2,G3 and G4 progression to ACLF



Fig. 4 Pronostic metabolic signature of the progression to ACLF enabling discrimination between G1,G2,G3 and G4 at admission

### CONCLUSION

The most interesting biomarker candidates were selected on the basis of the longitudinal follow-up of their concentrations over the 3-months period. Based on such metabolic signatures, we strive to identify new reliable biomarkers that could be further translated into point of care tests for improving cirrhotic patient care.

The most altered metabolic pathways were related to sugars and nucleosides, as previously published by our consortium (2). By focusing on the samples obtained at inclusion, we highlighted prognostic signatures of decompensation including 55 and 5 metabolites detected in serum and urine, respectively.

#### REFERENCES

(1) Trebicka et al. Journal of Hepatology 2021 (2) Moreau R. et al., Journal of Hepatology, 2020 (3) Giacomoni F. et al., Bioinformatics, 2015



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