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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ABSTRACT

Acute decompensation (AD) and its progression to acute-on-chronic 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.

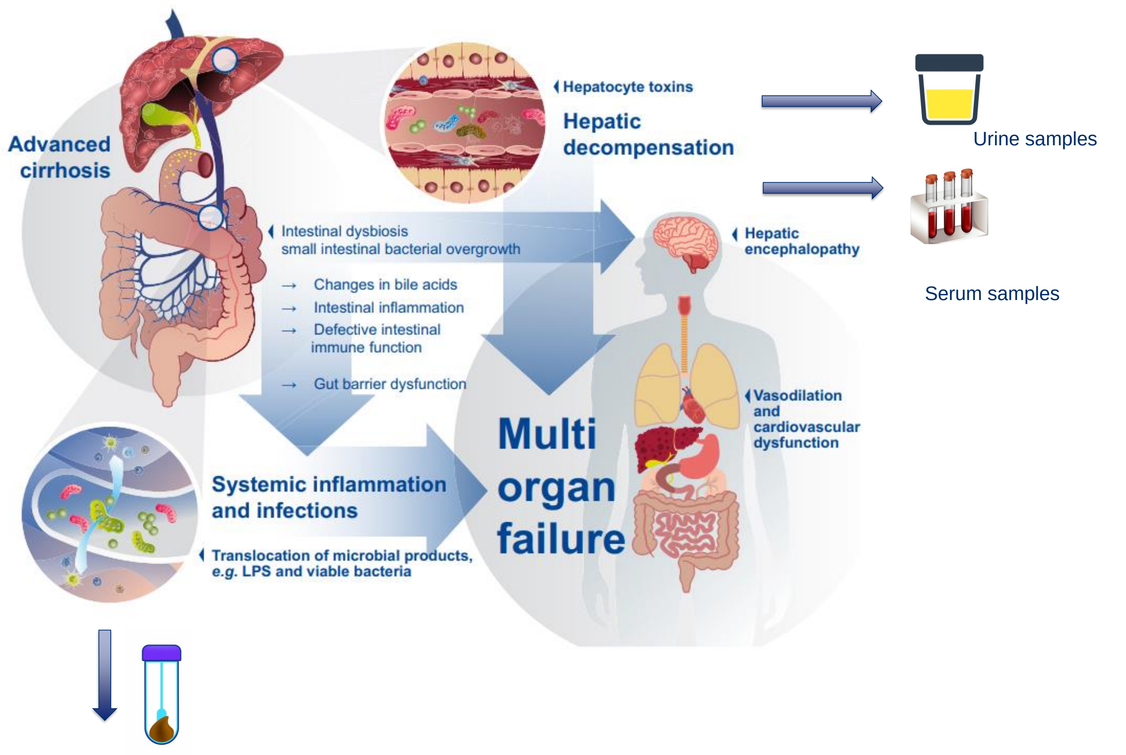


Fig. 1 Sampling hepatic decompensation (1)

METHODS

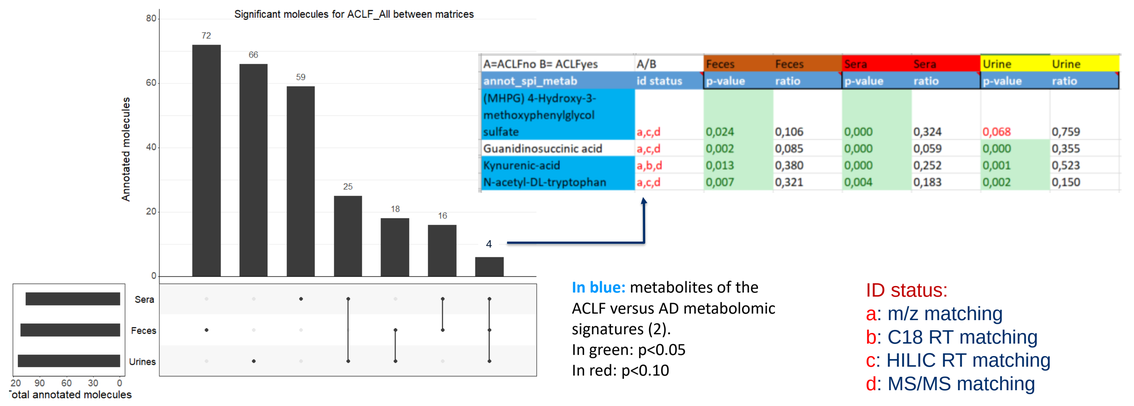
The untargeted metabolomic analysis of fecal, urine and serum samples (309 of each) collected from 94 patients over a 3-months follow-up period was performed using two complementary liquid chromatography coupled to high-resolution mass spectrometry methods (2). Datasets were pre-processed on the W4M 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.

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.

RESULTS

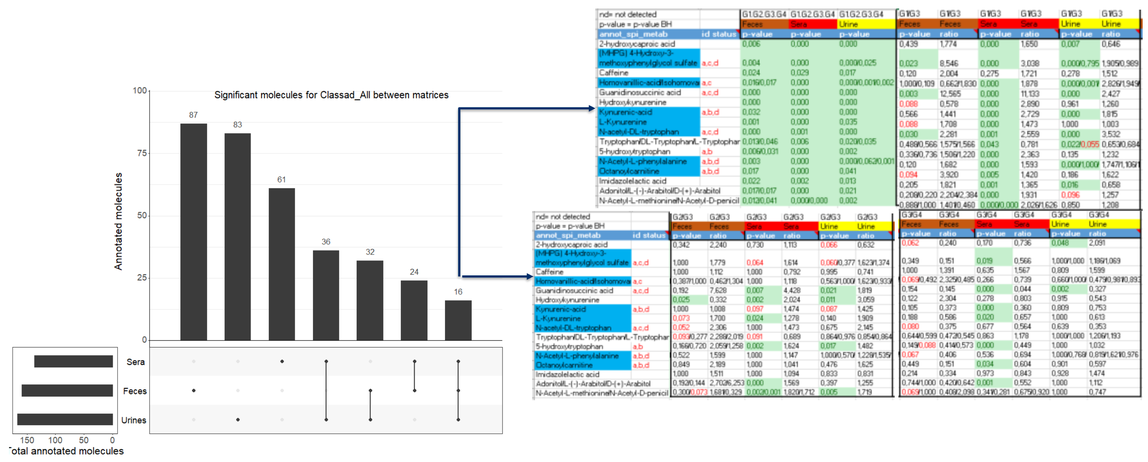


A=ACLFno B= ACLFyes	A/B	Feces	Feces	Sera	Sera	Urine	Urine
annot_spi_metab	id status	p-value	ratio	p-value	ratio	p-value	ratio
(MHPG) 4-Hydroxy-3-methoxyphenylglycol sulfate	a,c,d	0,024	0,106	0,000	0,324	0,068	0,759
Guanidinosuccinic acid	a,c,d	0,002	0,085	0,000	0,059	0,000	0,355
Kynurenic-acid	a,b,d	0,013	0,380	0,000	0,252	0,001	0,523
N-acetyl-DL-tryptophan	a,c,d	0,007	0,321	0,004	0,183	0,002	0,150

In blue: metabolites of the ACLF versus AD metabolomic signatures (2).
In green: p<0.05
In red: p<0.10

ID status:
a: m/z matching
b: C18 RT matching
c: HILIC RT matching
d: MS/MS matching

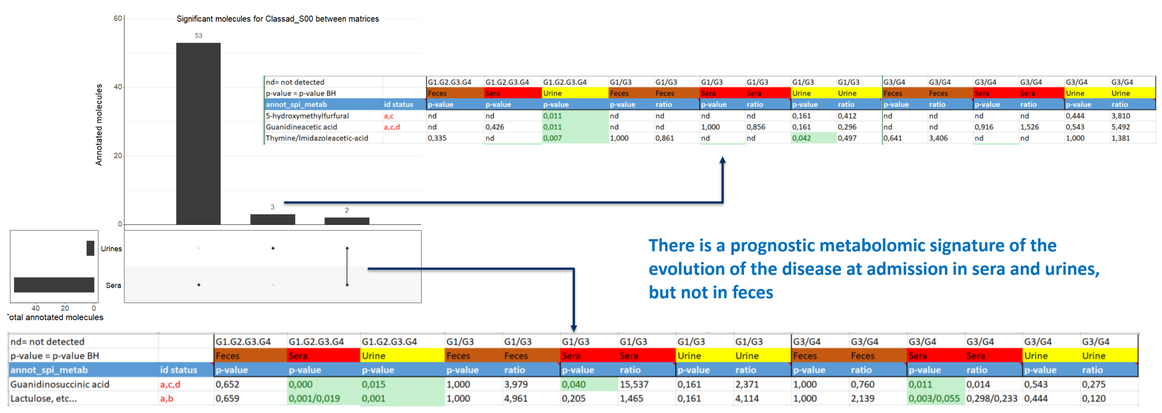
Fig. 2 Metabolic signature enabling to discriminate between ACLF (yes /no)



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)
G4: ACLF at admission

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

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



There is a prognostic metabolomic signature of the evolution of the disease at admission in sera and urines, but not in feces

nd= not detected		G1.G2.G3.G4	G1.G2.G3.G4	G1.G2.G3.G4	G1/G3	G1/G3	G1/G3	G1/G3	G1/G3	G1/G3	G3/G4	G3/G4	G3/G4	G3/G4	G3/G4	G3/G4
p-value = p-value BH		Feces	Sera	Urine	Feces	Feces	Sera	Sera	Urine	Urine	Feces	Feces	Sera	Sera	Urine	Urine
annot_spi_metab	id status	p-value	p-value	p-value	p-value	ratio	p-value	ratio	p-value	ratio	p-value	ratio	p-value	ratio	p-value	ratio
5-hydroxymethylfurfural	a,c	nd	nd	0,011	nd	nd	nd	nd	0,161	0,412	nd	nd	nd	nd	0,444	3,810
Guanidineacetic acid	a,c,d	nd	0,426	0,011	nd	nd	1,000	0,856	0,161	0,296	nd	nd	0,916	1,526	0,543	5,492
Thymine/Imidazoleacetic-acid		0,335	nd	0,007	1,000	0,861	nd	nd	0,042	0,497	0,641	3,406	nd	nd	1,000	1,381

nd= not detected		G1.G2.G3.G4	G1.G2.G3.G4	G1.G2.G3.G4	G1/G3	G1/G3	G1/G3	G1/G3	G1/G3	G1/G3	G3/G4	G3/G4	G3/G4	G3/G4	G3/G4	G3/G4
p-value = p-value BH		Feces	Sera	Urine	Feces	Feces	Sera	Sera	Urine	Urine	Feces	Feces	Sera	Sera	Urine	Urine
annot_spi_metab	id status	p-value	p-value	p-value	p-value	ratio	p-value	ratio	p-value	ratio	p-value	ratio	p-value	ratio	p-value	ratio
Guanidinosuccinic acid	a,c,d	0,652	0,000	0,015	1,000	3,979	0,040	15,537	0,161	2,371	1,000	0,760	0,011	0,014	0,543	0,275
Lactulose, etc...	a,b	0,659	0,001/0,019	0,001	1,000	4,961	0,205	1,465	0,161	4,114	1,000	2,139	0,003/0,055	0,298/0,233	0,444	0,120

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.

REFERENCES

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