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Highlights :

- Metabolomics refers to the study of biological components below 1000 Daltons (Da) involved in metabolic pathways as substrates, products or effectors.
- Untargeted metabolomics provide potential associations of metabolites to metabolic pathways and biochemical insights which can be further associated to a biochemical phenotype
- According to the pathophysiology of HE made from a close interaction between systemic inflammation and metabolic disturbances, especially hyperammonemia, metabolomics appears as a promising technique
- Recent studies suggest that metabolomics could unravel new HE pathophysiological pathways, enable the identification of new biomarkers and help in the prognostication and the prediction of treatment response
- Its wide acceptability and use needs to address some shortcomings: standardization in samples preparation and analysis, identification of the main confounders
- Some portable devices are in development and could lead to bring metabolomics to the bedside of HE patients.

Abstract (123 words)

Metabolomics refers to the study of biological components below 1000 Daltons (Da) involved in metabolic pathways as substrates, products or effectors. According to the interconnected metabolic disturbances that have been described in the pathophysiology of hepatic encephalopathy (HE), this technique appears to be well adapted to study and better delineate the disease. This review will focus on recent advances in metabolomics in the field of HE. Thus, after a brief overview of the general principles of metabolomics, we will discuss metabolomics as a potentially efficient tool for unraveling new HE pathophysiological insights, biomarkers identification, or as a predicting tool for treatment response or outcome prognosis. Finally, we will give our vision on the prospects offered by metabolomics for improving care of HE patients.

1. Introduction

Hepatic encephalopathy (HE) is defined as the neurological or the neuropsychological symptoms caused by an acute or chronic liver disease and/or a portosystemic shunt (1). Symptoms range from mild neuropsychological disturbances in the particular setting of minimal HE (MHE), only detected by extensive neuropsychological testing, or covert HE (MHE and also HE detected by caregivers), to confusion and coma in the setting of HE in its overt form (overt HE-OHE) (2). Although clinical HE is estimated to be present in about 10 to 15% of patients at the time of cirrhosis diagnosis, it is probably present in up to two-third of them during the disease course. The presence of HE is independently associated to increased mortality, and altered quality of life (3,4).

The two key features that have been recognized for years in participating in HE pathogenesis are hyperammonemia and systemic inflammation (see for review (5–7)). Due to altered liver function and portosystemic shunts, ammonia level increases in the systemic circulation. This increase could become even more important once the muscle mass decreases due to sarcopenia, as muscle cells express glutamine synthetase able to convert ammonia into glutamine. Ammonia can cross passively the blood-brain barrier and access the central nervous system where astrocytes, also expressing glutamine synthetase, convert it to glutamine from glutamate. Elevated levels of intracerebral glutamine probably largely contribute to astrocyte dysfunction and secondary altered neurotransmission (8,9). The role of concomitant alteration in gut metabolism with an increased glutaminase activity in the intestinal border cells and the possibility of increased translocation have been proposed as contributing factors for both hyperammonemia and systemic inflammation (10,11). More recently, modification in gut microbiota composition has been unveiled (12). Briefly, it appears

that intestinal microbiota diversity is decreased in cirrhosis and becomes even poorer in decompensated cirrhosis and in patients with HE, with an increase in pathogenic bacterial species, such as Proteobacteria, responsible for hyperammonemia, systemic inflammation and an increase in secondary to primary bile acids ratio. Important clues in favor of the major role of gut microbiota modifications have been brought up by two recent randomized controlled trials where the authors described that fecal microbiota transplantation could be able to reverse these phenotypes but also prevent further HE episodes (13,14). Despite these recent advances, many areas of the pathophysiology of HE are still not elucidated.

The “-omics” revolution has rapidly gained a widespread diffusion in the different domains of biology (15). In fact, the impact of these new biological techniques in the “-omics” era is huge and undeniable. The concept of “-omics” refers to technologies enabling the simultaneous measure, quantification and analysis of sets of genes (genomics), mRNA (transcriptomics), proteins (proteomics), or metabolites (metabolomics) in biological media thanks to data production tools such as nucleic acid sequencing, nuclear magnetic resonance spectroscopy or mass spectrometry, combined with data mining tools. It was first developed around genetic studies aiming at investigating genetic information as a whole. It comes with a renewed vision on biological questions and in particular pathophysiological mechanisms, aiming at an exhaustive picture of a biological phenomenon for a deeper understanding of the complex underlying biochemical mechanisms. Among these -omic approaches, the metabolome is thought to be the closest representation of the metabolic phenotype; and its large-scale study without any specific target constitutes the untargeted metabolomics. Untargeted metabolomics provide potential associations of metabolites to metabolic pathways and biochemical insights which can be further

associated to a biochemical phenotype. Therefore, metabolomics appears particularly interesting for studying HE owing to the interlinked metabolic disturbances.

This review will focus on recent advances in metabolomics in the field of HE. Thus, after a brief overview of the general principles of metabolomics, we will discuss metabolomics for getting deeper insight into HE pathophysiology, as well as a valuable tool for identifying biomarkers of disease progression, outcome prognosis or treatment efficacy. Lastly, future perspectives will be discussed.

2. General principles of metabolomics

Metabolomics refers to the large-scale study of biological components below 1000 Daltons (Da) involved in metabolic pathways as substrates, products or effectors (16–18). Its value to the field of HE can be anticipated owing to the aforementioned interlinked metabolic disturbances. It is based on the simultaneous analysis of hundreds to thousands of metabolites found in a biological sample, whether they are naturally present or produced by other biological systems such as microbiota in humans, or foreign to the biological system for example environmental compounds, xenobiotics or drugs (Figure 1). An overview of a typical metabolomic workflow is given in Figure 1. It starts with sample preparation and data acquisition steps, followed by data preprocessing, statistical analyses. An additional metabolite annotation/identification step is then performed as a prerequisite to further metabolite repositioning into corresponding metabolic pathways. Detailing all these steps is beyond the scope of this review. For greater detail, the reader is referred to recent review articles (17,19,20).

Metabolite detection essentially relies upon two types of techniques: nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). NMR spectroscopy is a non-destructive technique that can also provide accurate quantitative results, enabling the quantification of multiple metabolites with a single internal or external reference (19). In contrast, MS is a destructive but highly sensitive technique, therefore detecting many more metabolite-derived signals than NMR. Nowadays, liquid chromatography (LC) coupled to high-resolution and high-mass accuracy mass spectrometry (HRMS) with electrospray ionization (ESI) represents the most widely implemented approach for metabolomic purposes (21). LC offers efficient and flexible separation of metabolite mixtures while HRMS enables successful detection and identification thanks to high mass accuracy measurement of a large range of metabolites present at minute amounts in complex biological samples such as human biofluids (17).

One of the greatest challenges in untargeted metabolomics lies in the identification of metabolites. The identification process starts with data set annotation. Overall, such annotation process consists of associating a feature (i.e., a NMR chemical shift, an accurate *m/z* ratio measured by HRMS, or a [*m/z*, retention time] combination from LC-HRMS experiments) with those of a pure authentic standard analyzed under similar conditions. This can be achieved by interrogating in-house and publicly available biochemical, metabolomic and also spectral databases. For instance, in MS-based metabolomics, it is common practice to perform either MS/MS or sequential MSn experiments in order to confirm MS annotations. Combining NMR (1D, 2D, $^{1}H/^{13}C$) and LC-HRMS approaches can also greatly benefit to successful metabolite identification (19). Identified and/or annotated metabolites have then to be

replaced into metabolic pathways to further translate analytical data into valuable biological information (22).

Once pertinent metabolites and pathophysiological pathways have been identified or when specific hypotheses need to be addressed, targeted metabolomics enable simultaneous analysis of a large number of components and their study in regards to known pathophysiological pathways or clinical and paraclinical features.

3. Unraveling new HE pathophysiologic pathways

Both NMR- and MS-based metabolomics, by their unique characteristics without any previously established hypothesis, have been successfully applied in HE with promising results in animal models and in patients (Table 1). In animals, metabolomics has been performed in murine models of acute liver failure (ALF) and chronic liver disease (CLD). Several studies have suggested an impairment in energy metabolism with altered tricarboxylic acid cycle, glycolysis and ketogenesis (23–25). This seems to be a common feature of ALF and CLD models. These data also suggest the presence of regional metabolic differences in the brain that could explain differential clinical presentation (26). In humans, metabolomics also provides interesting pathophysiological insights. Table 1 depicts the main original studies on metabolomics in HE in both animal models and humans. Whereas the first major study by Jimenez et al. (27) used ^{1}H-NMR, almost all further studies involved MS-based approaches to obtain a broader metabolome coverage (28,29). Several studies focused on MHE patients who were well phenotyped by thorough neuropsychological testing. These patients presented with blood altered energy metabolism, decreased levels of branched-chain amino acids (BCAA) and fatty acids and increased levels of lactate and trimethylamin-N-oxide. In an original study,

McPhail et al. included a very wide clinical spectrum of cirrhotic patients with patients without HE, patients with covert HE and with OHE and studied urine metabolites (30). No data are available on brain tissue from humans but our group was able to analyze plasma and cerebrospinal fluid (CSF) samples of ICU hospitalized cirrhotic patients with or without HE (31). Interestingly, the analysis of CSF of cirrhotic patients presenting with HE has shed the light on some remarkable metabolic pathways involved in brain dysfunction. We found altered ammonia and amino-acids metabolism, encompassing increased glutamine levels, but also significant changes in energy metabolism. Clues in favor of an increase in the involvement of β-oxidation, an energetic pathway used by astrocytes but marginal in the brain in normal conditions, have been found. This could be secondary to the increase in ammonia and reduction of the Krebs cycle activity and could intervene in astrocyte dysfunction leading to altered neurotransmission regulation and neuronal homeostasis (31). Another key finding was the important amount of bile acids in the CSF, especially secondary bile acids, that were several hundred times higher compared to healthy controls. This was since confirmed in animal models of HE by others (32) and by us (unpublished data). Bajaj et al. found very similar results in a less severe population of cirrhotic outpatients by performing MS-based metabolomics on urine and blood samples (29). Abnormalities were found in key metabolic pathways such as energy metabolism, urea cycle, aromatic amino-acids and fatty acids metabolism that proved associated to outcome especially HE occurrence. Unexpectedly, about one third of the patients in our study displayed also several xenobiotics, *i.e.* drugs, in their CSF. While some substances like benzodiazepines or anticonvulsivants were somehow expected, the presence in the CSF of antimicrobial agents was unexpected. Indeed, those were given at a normal dosage and encompassed norfloxacine, fluconazole,

piperacillin or piperacillin/tazobactam. It could thus be discussed that, due to increased blood-brain barrier permeability in cirrhotic patients (33), some patients would display drug-induced encephalopathy in addition to classical HE.

Metabolomics has also been used to study further modifications of stool microbiota in cirrhotic patients with or without HE. Indeed, marked dysbiosis has been described in cirrhotic patients compared to healthy controls, and in cirrhotic patients with HE relatively to cirrhotic patients without HE (34). One of the main hypotheses is that this microbiota modification leads to a functional shift in microbiota metabolic pathways with especially a decline of bacterial autoregulation and network interactions (11). Moreover, Iebba et al. showed, by combining an analysis of stool and cecal microbiota through 16S-RNA on the one hand and metabolomics in peripheral and portal blood samples of cirrhotic patients on the other hand, that specific metabolic profiles (increase in faecal methanol and threonine and a decrease in faecal n-butyrate) as well as bacterial species and inflammation markers such as IL6 were significantly associated with the development of HE (11). Inflammatory disturbances in OHE and MHE patients were also found in a recent study combining transcriptomic, metabolomic and cytokine profiling in cirrhotic patients (28). Indeed, the authors were able to show in MHE patients that their adaptative immunophenotype tended to shift towards an auto-immune profile, that serum samples exhibited alterations of phospholipid metabolism and that inflammatory cytokines such as IL-6, IL-15 and IL-22 were significantly higher than in cirrhotic patients without MHE.

4. Identifying new biomarkers

The diagnosis of HE is frequently difficult. Whereas in overt HE the challenge is not to misdiagnose HE in the presence of a differential diagnosis and to grade appropriately the severity of the disease, in MHE, the challenge is more to identify HE with very few complaints from the patients and/or the caregivers. Metabolomics enables to identify specific metabolic profiles according to disease severity in an animal model of ALF (26). In humans, several studies showed the ability of metabolic profiles to distinguish cirrhotic patients without HE from cirrhotic patients with HE and this for both overt HE and MHE (27,31,35). Recently, metabolomics was applied to identify MHE in cirrhotic patients (27). Using two-dimensional NMR and internal databases to identify blood metabolites, the authors found that metabolomic profiles uncover MHE patients that were not well classified by clinical evaluation, especially outlier patients, *i.e.* for whom psychometric tests results from PHES and CFF were discordant (out-of-the-box patients) by using blood metabolites. Thus, patients with MHE displayed increased serum concentrations of glucose, lactate, methionine, trimethylamin-N-oxide (TMAO), and glycerol, and decreased levels of choline, BCAA, alanine, glycine, acetoacetate, α-acid glycoproteins, and lipid moieties. Using urine metabolites, McPhail et al. were able to isolate a specific metabolomic pattern of patients with OHE, when comparing them to patients without HE. However differentiating covert HE from no HE was not possible in this study (26). We obtained very similar results by using CSF-metabolomic data from plasma samples, to distinguish cirrhotic patients with or without overt HE (31). Nevertheless, this clear difference between patients with or without HE was mainly found by using supervised partial least-squares – discriminant analysis (PLS-DA) and less frequently by using unsupervised principal component analysis (PCA). Furthermore, all those distinctions

take into account tens of metabolites that are not easily transferable to simple biochemical routine dosages. Another limitation is the presence of potential confounding factors in those profiles, especially external factors such as diet, intoxications, medication, and age. It should be noticed also that, in the most severe patients, the metabolomic signature of OHE could be confounded by an ACLF signature. In the future, more extensive data on large amounts of well characterized patients, in blood, urine but also CSF samples, could confirm these findings and allow to restrict the list of metabolites to a "short-list" of metabolites candidates assessable in routine. Another opportunity would be the development of portable devices able to bring metabolomic techniques to bedside (see below Perspectives).

5. Prognostication and prediction of treatment response

Another promising application of metabolomics could be prognostication, *i.e.* predicting in a cohort of patients those who are at high risk of developing HE or those that will respond, or not, to a given treatment option. Two recent studies showed very promising results in patients with liver decompensation regardless of the presence of HE (29,36). Using ^{1}H-NMR and LC-HRMS on plasma samples obtained from cirrhotic patients admitted for acute decompensation, McPhail and al. were able to accurately predict survival (AUC 0.96), and treatment response (36). Quite similar results were recently found to distinguish the different acute-on-chronic liver failure (ACLF) grades in an ancillary study of the CANONIC cohort (37), giving support to the validity of such findings. Despite no direct relation with HE, those results are important since patients displaying ACLF present more frequently OHE and since ACLF and HE metabolomic signatures could be interlinked. More recently, Bajaj et al. showed that metabolomics analyses performed on urine and blood samples of cirrhotic

outpatients were able to predict HE occurrence, hospital admission, need for transplant and death (29). Furthermore, urine and serum metabolomic profiles tended to be better predictors than MELD score (29). Thus, metabolomics could help in the early identification of “at high-risk” patients, consequently selected for an intensified follow-up.

Finally, some preliminary data suggest that metabolomics could help in the prediction and the evaluation of HE treatment response. Barba et al. were able to show a correlation between metabolomic profiles and HE outcome in an experimental model of therapeutic hypothermia in ALF (26). More recently, Bajaj et al. used metabolomic and stool microbiota analysis to evaluate the effect of rifaximin in HE in cirrhotic patients (34). They found no significant modification in microbiota but an increase in serum saturated and unsaturated fatty acids after treatment with rifaximin.

6. Perspectives

The main limitation of the wide use of metabolomics is still the accessibility to the technique and the expertise associated with its use and data interpretation. This could be resolved in a near future. Indeed, LC/MS is more and more accessible in clinical routine in tertiary care centers especially for toxicology (38). The technique itself relies upon necessary standardized procedures given the complexity of analyses. However, standardized procedures would facilitate large-scale reproducible studies and thus diffusion in routine analysis (39). Even though the “best” sample has long been considered the invasive one such as blood, CSF or ascite, several data suggest good performance for urine, sweat and even exhaled air. Some authors suggest a “real-time” bedside use of the technique in order to achieve direct implication in diagnosis, therapeutic response and prognosis. Portable devices

able to analyze exhaled air are under development, like the “electronic-nose” (40). Such devices could really enable widespread use of the technique, although their sensitivity and quantitative performances has still to be established. We previously saw that metabolomics was able to unravel dysfunctional metabolic pathways in HE. Even if these findings rely mainly on observational correlations and not causality, the data provide some clues for both drug repositioning and specific drug development. Indeed, the functionality of some of these metabolomic pathways can be affected by drugs that are already available. It is also possible to identify, *in silico*, drugs able to modulate these metabolic pathways. Finally, those *in vitro* metabolomic techniques could be complementary to other *in vivo* techniques like magnetic resonance spectroscopy (MRS). Thus, Braissant et al. showed, in an animal model of HE, that by using high field MRS they were able to identify and distinguish between metabolites inside the brain of the animals (41). These techniques are available in routine for humans at lower fields (3T) and showed promising results (42). Combination of these different techniques is a promising approach.

7. Conclusion

In summary, metabolomics provides numerous solutions to complex interrogations in the understanding, diagnosis, treatment and prognosis of HE. It remains a research technique but exponential increase of publications on its clinical applications reflects the possibilities of a shift towards routine uses. Indeed, metabolomics offers an original snapshot of pathophysiological processes in HE at a given moment. Moreover, metabolomic profiles are relevant in non-invasive samples such as urine, due to the systemic distribution of metabolic products and effectors and the systemic consequences of metabolic disturbances. In this context, an interesting perspective

could be to use data mining and statistical tools to combine metabolomics data obtained from different kinds of biofluids (*i.e.*, blood, urine and CSF) on the same patients, and to compare them to results obtained on HE animal models, the objective remaining the distinction between molecular signatures coming from liver injury, associated systemic inflammation, and those related to alteration of brain metabolism. It should however be noted that in the future, metabolomic studies should be ideally prospective and encompass very precise selection of the patients, detailed protocols for biofluid collection and storage in order to minimize confounders.

Easily obtainable samples, rapidly obtained results and help from robust statistical tools characterize metabolomics, as well as the systematic biological relevance of what is highlighted. We foresee in this the possibility of personalized and precise medical care at the patient's bedside.

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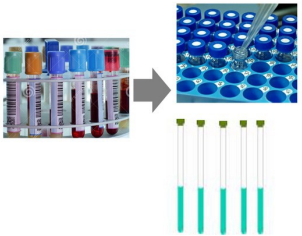
Legends:

Table: Main original studies on metabolomics in hepatic encephalopathy in both animal models and humans.
** corresponds not exactly to metabolomics $ reanalysis of previously acquired data using a specific tool*

Abbreviations: ^{1}H, proton; ALF, acute liver failure; BLD, biliary duct ligation; BCAA, branched chain amino acids; CLD, chronic liver disease; CSF, cerebrospinal fluid; GC, gas chromatography; HE, hepatic encephalopathy; HPLC, high performance liquid chromatography ; LC, liquid chromatography; LT, liver transplantation; MHE, minimal hepatic encephalopathy; MS, mass spectrometry; NMR, nuclear magnetic resonance.

Figure: General principles of metabolomics.
General principles of metabolomics are based of several successive steps: 1, the biofluids of interest has to be collected and then prepared in adapted samples ; 2, the samples have to be analyzed by GC/MS, LC/MS or RMN ; 3, a preprocessing step enables to select relevant signals among automatically detected signals and to compare them to internal and public databases ; 4, a statistical analysis step will enable to distinguish difference in signals or signatures according to different predetermined groups ; 5, annotations have to be confirmed and unknowns characterized in order to identify all the metabolites ; 6, a final step will enable to visualize findings in metabolic maps.
Abbreviations: GC, gas chromatography; gp, group ; LC, liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance ; RT, retention time.

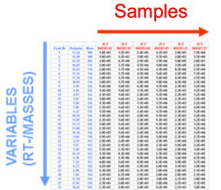
1. Sample preparation



2. Instrumental analysis

GC/MS or LC/MS or NMR

3. Data pre-processing

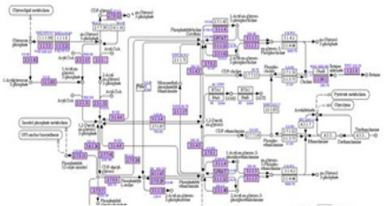


Automatic detection of signals

Selection of relevant features

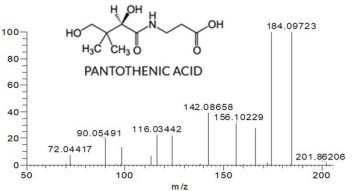
Annotation :
Public databases
Internal databases

6. Data visualization



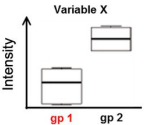
5. Metabolite identification

- To confirm annotations
- To characterize unknowns



4. Statistical analyses

Multivariate



Univariate

	Technic(s)	Study design and main findings	Key topic
Animal			
Meredith et al. 1986	Gaz ionization /MS *	Rat model of ALF/CLD (portosystemic bypass model), n=n.a., brain samples *Increased levels of octopamine and tyramine in the brain.*	Pathophysiology
Murphy et al. 2007	MS-MS	Mice model of ALF (surfactant/virus model), n=9-11 per group, blood samples *Increased levels of both medium- and long-chain acylcarnitines (C6:0–C12:0 and C14:0–C20:0, respectively).* *Clues in favor of diffuse mitochondrial dysfunction.*	Pathophysiology
Barba et al. 2008	^{1}H NMR	Rat model of ALF, n=6 per group, brain samples *Different profiles in the brain according to HE grades enabling severity scoring. Regional discrepancies in metabolic deterioration (brainstem earlier affected than cortical regions). Lesser abnormalities in treated animals.*	Grading (biomarker) Treatment follow-up (hypothermia)
Bak et al. 2009	MS *	Rat model of CLD (BDL), n=n.a., blood, liver, muscles and brain samples *Increased metabolism of isoleucine in muscles compared to the brain suggesting that therapeutic effect of BCAA is mainly due to an effect in the muscle and seems not able to bypass tricarboxylic acid cycle in the brain.*	Pathophysiology
Marini et al. 2017	HPLC & targeted LC/MS	Rat model of CLD (ammonium enriched diet) n=5 per group, brain dialysates *Lower baseline levels of adenosine, taurine, hydroxy-proline, and acetylcarnitine in hyperammonemic animals and higher levels of putrescine.* *Higher increase in adenosine after sleep deprivation in hyperammonemic animals and different time-course compared to control animals.*	Pathophysiology
Pathania et al. 2020	^{1}H NMR	Rat model of CLD (BDL) n=5 per group, liver and brain samples *Increased levels of lactate, acetate, succinate, citrate, and malate in the brain whereas the levels of glucose, creatine, isoleucine, leucine, and proline were decreased. Increased levels of lactate, BCAA, glutamate, and choline in the liver, whereas levels of glucose, phenylalanine, and pyridoxine were decreased.* *Altered energy metabolism (tricarboxylic acid cycle, glycolysis, and ketogenesis) in both the liver and the brain.*	Pathophysiology
Human			
Amuro et al. 1981	GC/MS *	Cirrhotic patients, n=13 (2 HE patients, comatose) *Possible abnormal keto bile acids levels in urine and stool*	Pathophysiology
Goldberg 1981	GC/MS *	HE patients, n=18 (13 grade 1-2 and 5 grade 3-4) compared to control patients, n=20 (6 healthy controls, 5 patients hospitalized for colonoscopic exploration, 4 cirrhotic patients without HE, 5 with portocaval shunts) *Increased levels of 3-methylbutanal in HE patients compared to controls that correlated to grades of HE.*	Pathophysiology Grading (biomarker)
Jimenez et al. 2010	^{1}H NMR	Cirrhotic patients, n=101 (no MHE=62, MHE=39) vs 69 healthy controls *Increased serum levels of glucose, lactate, methionine, trimethylamin-N-oxide, and glycerol in MHE patients and decreased levels of choline, BCAA, alanine, glycine,*	Pathophysiology Biomarker

		acetoacetate, alpha-acid glycoproteins and lipids.	
Bajaj et al. 2013	GC&LC/MS	MHE cirrhotic patients, n=20 Baseline and 8 weeks after rifaximin *Increase in serum saturated (myristic, caprylic, palmitic, palmitoleic, oleic and eicosanoic) and unsaturated (linoleic, linolenic, gamma-linolenic and arachnidonic) fatty acids after treatment with rifaximin, no modification in microbiota.*	Pathophysiology Treatment follow-up (rifaximin)
Weiss et al. 2016	LC/MS	CSF samples in 14 HE patients and 27 controls Serum samples in 13 cirrhotic patients without HE, 12 cirrhotic patients with HE and 9 controls *Increased CSF levels of amino acids, acylcarnitines, bile acids and xenobiotics (drugs) in HE patients.* *Accumulation of acetylated compounds could be due to a defect of the Krebs cycle in HE patients.*	Pathophysiology Biomarker
McPhail et al. 2017	^{1}H NMR	Cirrhotic patients, n=52 and 17 healthy controls 45 patients were well-characterized for the presence of HE (No HE=17, covert HE=7, overt HE=21) *Different metabolic profiles enabled to distinguish patient with overt HE from patient without HE but could not distinguish patient according to different HE grades or covert HE from patient without HE.*	Biomarker
Lebba et al. 2018	^{1}H NMR	Cirrhotic patients, n=46 and 14 healthy controls *Fecal methanol and threonine levels were positively associated with HE whereas n-butyrate levels were negatively associated with HE.*	Pathophysiology
Bajaj et al. 2019	LC/MS	Cirrhotic patients, n=211 (hospitalized=64, HE=19, LT=13, death=11) *Altered energy metabolism and bile acids.* *HE associated with 284 metabolites in urine and 228 in serum* *Prognostic value better than MELD*	Pathophysiology Outcome
Takado et al. 2019	^{1}H NMR & LC/MS	Healthy adults, n=20 Glutamine challenge *Glutamine levels determined by ^{1}H NMR in the brain (posterior cingulate cortex, i.e. a major hub of the default mode network) & LC/MS in the blood were correlated whereas glutamate levels were not.*	Biomarker Treatment follow-up
Frainay et al. 2019	MetaboRank tool for secondary analysis $	Reanalysis of samples of Weiss et al. with MetaboRank *alpha-ketoglutaramate is proposed to be added as a metabolic fingerprint of HE*	Pathophysiology
Rubio et al. 2021	Multi-omic approach LC/MS-MS	Cirrhotic patients, n=11 (no MHE=5, MHE=6) *Suggested link between long-chain unsaturated phospholipids and the increased fatty acid transport and prostaglandin production.*	Pathophysiology

Table: Main original metabolomics-based studies in hepatic encephalopathy in both animal models and humans.

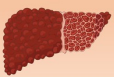
** correspond not exactly to metabolomics $ reanalysis of previously acquired data using a specific tool*

Abbreviations: ^{1}H, proton; ALF, acute liver failure; BLD, biliary duct ligation; BCAA, branched chain amino acids; CLD, chronic liver disease; CSF, cerebrospinal fluid; GC, gazeous chromatography; HE, hepatic encephalopathy; HPLC, high performance liquid chromatography; LC, liquid chromatography; LT, liver transplantation; MHE, minimal hepatic encephalopathy; MS, mass spectrometry; n.a., not available ; NMR, nuclear magnetic resonance.s

Metabolomics on its way to the bedside for hepatic encephalopathy patients ?

Hepatic encephalopathy

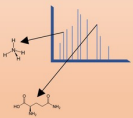
Metabolic disturbances
(hyperammonemia, …)
Inflammation



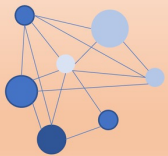
Metabolomics

Invasive and non invasive samples

In vivo or *ex vivo* analysis



Identification



Interpretation