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Admission Serum Metabolites and Thyroxine Predict Advanced Hepatic Encephalopathy in a Multi-center Inpatient Cirrhosis Cohort

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Abstract

Background and Aims: Grade 3–4 hepatic encephalopathy (advanced HE), also termed brain failure (BF), is an organ failure that defines acute-on chronic liver failure. It is associated with poor outcomes in cirrhosis but cannot be accurately predicted. We aimed to determine

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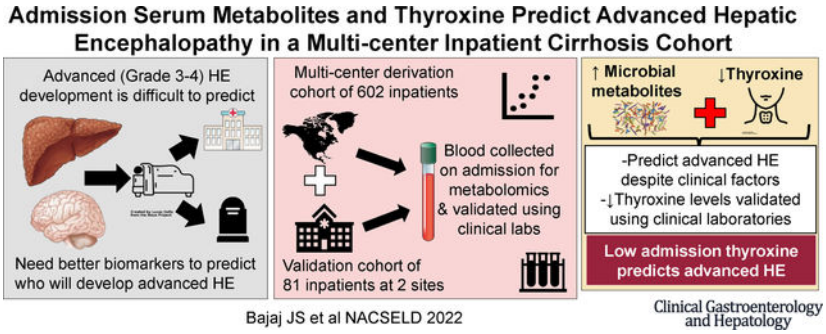
the admission metabolomic biomarkers able to predict the development of advanced HE with subsequent validation.

Methods: Prospective inpatient cirrhosis cohorts (multi-center and 2-center validation) without BF underwent admission serum collection and inpatient follow-up. Serum metabolomics was analyzed to predict BF on random forest analysis (RFA) and logistic regression. A separate validation cohort was also recruited.

Results: The *multi-center cohort* included 602 patients, of whom 144 developed BF (105 only BF) 3 days post-admission. Unadjusted RFA showed that higher admission microbially-derived metabolites and lower isoleucine, thyroxine and lysophospholipids were associated with BF development (AUC 0.87 all, 0.90 BF only). Logistic regression AUC with only clinical variables significantly improved with metabolites (0.65 to 0.75; p=0.005). Four metabolites that significantly added to BF prediction were low thyroxine and maltose and high methyl-4-hydroxybenzoate sulfate and 3–4 dihydroxy butyrate. Thyroxine alone also significantly added to the model (p=0.05). *Validation cohort*: prospectively included 81 patients, of whom 11 developed BF. Admission hospital laboratory thyroxine levels predicted BF development despite controlling for clinical variables with high specificity.

Conclusions: In a multi-center inpatient cohort, admission serum metabolites, including thyroxine predicted advanced HE development independent of clinical factors. Admission low local laboratory thyroxine levels were validated as a predictor of advanced HE development in a separate cohort.

Graphical Abstract



Keywords

brain failure; acute-on-chronic liver failure; thyroid; metabolomics; outcomes

INTRODUCTION:

Inpatient hepatic encephalopathy (HE) management requires a rapid and flexible strategy to protect the airway, correct precipitants, and initiate HE-specific therapy[1]. However, developing advanced HE (grades 3–4 HE) necessitates transfer to monitored units for airway protection and if not anticipated or treated in time, can result in aspiration pneumonia, and need for intubation[1, 2]. The prognosis of patients in advanced HE is often worse than earlier grades, and is considered “brain failure” in acute-on-chronic liver failure (ACLF)

definitions.[3–5]. Also, the development of advanced HE complicates transplant candidacy[5]. Current studies have focused on the outcomes after advanced HE development, but it is unclear which subgroup of inpatients will develop this complication. Identification of this subgroup could improve prognostication, encourage transfer to monitored settings, such as intensive care units (ICU), reduce chances of aspiration pneumonia and falls, and reduce the time before second-line therapies are initiated[2]. Metabolomics, or the analysis of metabolites in a sample, is a promising approach to biomarker discovery, especially in easily collectable samples such as serum[6–8]. Metabolomics has been used in cirrhosis and may be important to improve detection of complications and improve prognosis[9–11].

Our aim was to determine if admission serum metabolites can predict the development of advanced HE in patients with cirrhosis admitted without this complication and validate them in a separate inpatient cirrhosis cohort.

MATERIALS AND METHODS:

Multi-center cohort:

North American Consortium for the Study of End-Stage Liver Disease (NACSELD-2) cohort consists of prospectively recruited cirrhosis inpatients from multiple North America centers. Cirrhosis was defined by liver biopsy, evidence of varices on endoscopy or imaging or thrombocytopenia in chronic liver disease patients, or prior or current decompensation.

Consent was obtained from patients or representatives. We included only patients with confirmed cirrhosis were admitted non-electively, without advanced HE or ACLF on admission and could provide serum within 12 hours of hospitalization. Patients with unclear cirrhosis diagnosis, HIV, admission ACLF or advanced HE, or prior organ transplants and those in whom samples could not be obtained, were also excluded.

We recorded demographics, cirrhosis details, admission laboratory values, inpatient course including infections, organ failures, ACLF (NACSELD criteria), ICU transfer and death. HE was defined using the West-Haven criteria using local PI assessment[12].

Serum was collected, stored at −80 degrees, and sent for analysis using published LC/MS metabolomics to Metabolon Inc (Morrisville, NC, supplement)[13]. We first performed ANCOVA metabolite analysis adjusted for admission MELD, Sodium (Na), albumin, WBC, age, gender, alcohol-related etiology, rifaximin use and infection status to determine differences between groups. Subsequently a random forest analysis (RFA) was performed between those who developed advanced HE or not based on admission metabolomics expressed as mean decrease in accuracy (MDA) for the top metabolites.

Finally, logistic regression models for advanced HE were developed for the base model and then for the base model plus metabolites that were significant on RFA. The base model was created using age, WBC, Na, Albumin, rifaximin, prior HE, infection, and MELD at the time of admission. The serum metabolites that were statistically significant on RFA were then added to this base model. From these models, receiver operator characteristic (ROC) curves and the areas under these curves (AUC) were created, as well as their 95%

confidence intervals were calculated. The AUC values for the base model and the base plus metabolite models were compared using the non-parametric method of DeLong for two or more correlated ROC curves[14]. Finally, we performed the net reclassification index (NRI) analysis to determine the impact of metabolites over the clinical model.

Validation cohort:

A separate group of inpatients with cirrhosis with similar eligibility criteria as the NACSELD-2 cohort was enrolled in Richmond VA and VCU medical centers. Consent was obtained from patients or legally authorized representatives and patients were followed for development of advanced HE during the hospitalization. All eligible subjects were free of ACLF or advanced HE on admission and provided serum. The serum was stored at −80 degrees and used for local validation testing at the CLIA-certified laboratory at Richmond VAMC. Significant metabolites in the multi-center cohort that could be analyzed locally were evaluated to determine differences between those who developed advanced HE or not with multi-variable logistic regression was also performed using clinical data and metabolites (Figure 1). The IRBs at all sites approved the protocol before study activities were initiated.

RESULTS:

Patient Characteristics:

Derivation cohort: 602 inpatients of which 24% (n=144) developed advanced HE median 3 (2–8 days IQR) days after admission (Table 1). Of these, the majority (N=105) had isolated advanced HE without other organ failures. Patients who developed advanced HE had higher MELD scores, disease severity by laboratory values, admission rifaximin use, and percent with infection on admission but a similar percent with prior TIPS. The profile of infections was largely similar, although there was a higher rate of spontaneous bacterial peritonitis and urinary tract infections in those who developed advanced HE versus not (Supplementary table 1). Only twelve patients had current alcohol misuse on admission. There was a higher rate of admissions for GI bleeding, AKI, anasarca, and patients with prior HE without infection in those who developed advanced HE. This translated into a higher rate of ACLF and other organ failures, length of stay, ICU transfer, and inpatient death compared to patients who did not develop advanced HE.

Metabolomics:

Using RFA the AUC for all advanced HE prediction was 0.87 and the thirty highest metabolites on RFA and LS-means in figures 2 and 3. Similar metabolites were found for the advanced HE only patients with AUC of 0.90. Thyroxine (total thyroxine), isoleucine and lysophospholipids were lower but potential microbial metabolites i.e., aromatic amino acids and benzoate metabolites, were higher in those who developed advanced HE, regardless of whether in combination with other failures or alone. Especially since >72% of patients developed isolated advanced HE as the only organ failure. The specific directions of change are also shown in table 2 and least-squares means in figures 2 and 3 with fold changes using ANCOVA are in tables S2.

Logistic regression: We used the clinical model for logistic regression for the prediction of advanced HE using age, gender, MELD score, serum Na, WBC count and albumin levels, admission infection and prior HE. The AUC for prediction using the clinical model was 0.65 (95% CI 0.60–0.70), which was improved after adding metabolites that were significant on RFA (Table 2) to an AUC of 0.75 (95% CI 0.68–0.79). This improvement was statistically significant at p=0.0005 and involved four admission metabolites (1) thyroxine (lower), (2) methyl-4-hydroxybenzoate sulfate (higher), (3) 3–4 dihydroxy butyrate (higher), and (4) maltose (lower). Using only thyroxine as the metabolite added to the clinical model, there was again a significant increase in prediction of the AUC to 0.72 (0.63–0.75) with p=0.05 compared to the clinical model alone. The NRI was 0.1219 (95% CI: 0.0562, 0.1877) which is significantly different from the clinical model alone (0) (z = 3.64, p = 0.0003).

Given the findings above, the ability of clinical laboratories to perform thyroid hormone levels in clinical laboratories, we focused on validating whether this is lower in those who developed advanced HE in a separate validation cohort.

Validation cohort:

Patient cohorts: Eighty-one patients admitted at VCU/Richmond VAMC (59 men, MELD 17.5±8.9) for infections (n=24), ascites or anasarca (n=18), acute kidney injury (n=16), HE (n=16), other liver-related causes (n=8) and liver-unrelated causes (n=9) were included. None had advanced HE on admission. Eleven patients developed advanced HE a median of 5.3±2.2 days post-admission. None were on thyroid medications, while 41 had prior HE and 27 were on rifaximin therapy. Patients who developed advanced HE had a higher rate of grade 1–2 HE on admission while other factors, including prior TIPS, were not significant (Table 3).

Thyroid hormone levels: Using the clinical laboratory, we found significantly lower admission total thyroxine and Free (FT4) levels and higher thyroid uptake in those who developed advanced HE versus not while TSH levels were statistically similar(Table 3). Using FT4 levels, the AUC to predict advanced HE was 0.72 (95% CI 0.57–0.88, p=0.02), while for total thyroxine was 0.74 (0.59–0.89, p=0.01). Since we were aiming for greater specificity, a cut-off of >0.72 uIU/mL of FT4 gave 95.7% specificity and 37% sensitivity while a cut-off of >4.3 ug/dl of total thyroxine had 91.4% specificity and 37% sensitivity.

On multi-variable logistic regression, low thyroxine (OR 0.67, CI: 0.48–0.89, p=0.01) and presence of grade 1–2 HE on admission (OR 7.32, CI 1.68–19.43, p=0.008) were significantly predictive of development of advanced HE.

DISCUSSION

We found that serum metabolites focused on microbially-generated products and low thyroxine were associated with the risk of subsequently developing advanced HE either alone or in combination with other organ failures in a large prospective multi-center inpatient cirrhosis cohort, which was independent of clinically available demographics and characteristics. Ultimately, despite concomitantly present organ failures, infections and adjustments for cirrhosis-related medications, low thyroxine levels on admission were

unique to the prediction of advanced HE development. Further, low admission thyroid hormone levels run in a local laboratory were then also associated with an increased risk for advanced HE development in an independent inpatient cirrhosis cohort.

Development of advanced HE represents a major change in the natural history of inpatients with cirrhosis that is difficult to predict using current clinical prediction tools[3, 5]. This was also underscored by the modest AUC for the ROC curve generated through logistic regression of clinical factors alone. This can result in aspiration pneumonia, falls, infections and precipitate a cycle of readmissions if not prevented or treated quickly [1]. There remains room for improvement in the management of these patients even in tertiary care centers [2]. Therefore, refining prediction is needed and the addition of metabolomics could be used as a means towards this goal. However, the role of the individual metabolites needs to be studied in the context of (a) association with brain dysfunction and gut-brain axis, (b) potential specificity for advanced HE vis-à-vis generalized liver dysfunction and other organ failures, and (c) additive value over current clinical biomarkers for brain failure prediction.

Overall, since most patients who developed advanced HE actually had this as their only organ failure, the AUC for prediction with metabolites was similar regardless of advanced HE plus other organ failures or advanced HE alone in this context. Alteration of the gut-brain axis with metabolites related to bacteria are associated with the pathogenesis of HE and brain failure[15]. Microbial composition on admission for patients who subsequently developed advanced HE versus those who do not is enriched with higher Enterobacteriaceae and lower Fusobacteriaceae[16], but analysis of their products may be more important[17]. Therefore, it is interesting that several major serum metabolites of microbial origin were different on admission between patients who did vs did not go on to develop advanced HE, and two of them (3,4-dihydroxybutyric acid and methyl-4-hydroxybenzoate sulfate) persisted in predicting this outcome despite adjustment for clinical factors. Since most patients did not provide stool, we were not able to link these specific metabolites with the microbiome; however, there have been prior studies linking these metabolites with bacterial and non-human metabolic processes. Several of these metabolites are tryptophan, benzoate, and polysaccharide fermentation products of bacteria[18]. 3,4-dihydroxybutyric acid is associated with *E.coli* and is formed through degradation of di- and polysaccharides[19, 20]. Accumulation of 3,4-dihydroxybutyric acid results in multiple neuromuscular deficits and is associated with brain dysfunction[21]. 3,4-dihydroxybutyric acid is an intermediate in GABA metabolism, which is also implicated in HE pathogenesis. 7alpha-Hydroxy-3-oxo-4-cholestenoate is involved in the primary bile acid biosynthesis pathway and is a measure of blood brain barrier permeability and neurodegeneration[22, 23]. Methyl-4-hydroxybenzoate sulfate, which is a widespread benzoate and xenobiotic metabolite, has been associated with the impact of alcohol on the brain through phytochemical modification[24]. Our major etiology was alcohol-related cirrhosis but not active alcohol intake, and as such this is unlikely to be due to alcohol intake *per se*. Therefore, several metabolites have been associated with brain dysfunction in patients with and without cirrhosis, which enhances the biological plausibility of these results.

In addition to the linkage with brain dysfunction, we evaluated the potential specificity towards advanced HE, which overall was defined by similar AUCs regardless of HE being

the only organ failure or not. However, few metabolites such as lower phospholipids and branched chain amino acids, and higher methyl-4-hydroxybenzoate sulfate and phenyllactate overlapped between advanced HE and overall ACLF development as reported in our cohort and others previously[7, 13, 18, 25, 26]. Therefore, these selected metabolites (phospholipids, branched chain amino acids, phenyllactate and methyl-4-hydroxybenzoate sulfate) are likely a reflection of liver disease progression over time rather than specific for advanced HE.

It is important to reiterate that the remaining metabolites are distinct from prior published studies that evaluated metabolomics in established ACLF and those that predicted the need for dialysis or development of acute kidney injury[11, 27, 28]. Moreau and Claria et al found changes focused on energy metabolism and the specific kynurenine pathways in patients who had already established ACLF compared to others, while those who developed AKI and needed dialysis had a specific profile of uremic toxins in blood which does not overlap with these metabolites significantly[11, 27, 28].

The most accessible result of these was the low thyroxine levels in serum of patients who developed advanced HE, which significantly added to the ROC prediction in the derivation cohort beyond the clinical model. Given the metabolomic analyses performed, specific quantitation was not possible in the derivation cohort, but the fold-change and direction of changes can be gleaned. Therefore, to translate this potentially into practice, a validation cohort using the local clinical laboratory was needed. We chose this thyroxine levels as a proof of concept for the validation cohort rather than the other three because most clinical laboratories can readily perform this assay. The association of low thyroid hormone levels with fatty liver and cirrhosis has been described in outpatients and inpatients but often this is related to the underlying liver disease [29, 30]. Hypothyroidism is often a mimic for HE and there is evidence that this may precipitate hyperammonemia[31, 32]. The relatively low T4 levels are likely specific for advanced HE rather than just overall sickness because in the same population where renal failure and mortality was the outcome, thyroxine levels were not significantly predictive[11, 33]. Moreover, thyroxine levels were associated with development of advanced HE despite controlling for clinical data and was independent of grade 1–2 HE on admission. Similar patterns of thyroxine levels between our larger multi-center cohort using LC/MS and the smaller validation cohort using standard clinical techniques point towards a major prognostic role of hospital admission thyroxine levels.. This indicates a role of FT4 as a prognostic factor for advanced HE which could be linked with altered microbiota and immune dysfunction[34]. These processes could then predispose towards further brain dysfunction and alterations in mental status leading to advanced HE.

The results are clinically relevant because unlike prior metabolomic studies, which do not have a rapid clinical throughput, we found similar findings of lower total thyroxine in a separate smaller cohort using our local laboratory. However further external validation is needed after which, these levels could be used to triage patients at greatest risk for development of advanced HE to: (i) be monitored more closely, (ii) have a lower threshold for initiating second-line therapies and (iii) earlier transfer to a higher level of monitoring[35]. As also mentioned above these metabolites are likely specific for advanced HE development because our prior studies in this cohort showed differing metabolites

that predict ACLF, death or kidney-related outcomes,[11, 13] and they are associated with neurological impairments in other disease processes.

Our study is limited by its cross-sectional analysis of serum metabolites in a tertiary-care setting, which may not be applicable at other sites. We did not study ammonia or inflammatory markers, which would also be interesting as part of future studies[36, 37]. We were also unable to evaluate the impact of sarcopenia, minimal HE, and porto-systemic shunts, which have been associated with outpatient HE development in outpatients[38, 39]. The diagnosis of advanced HE was made by the local PIs according to best clinical practice and other causes of altered mental status could have been missed[1]. We only used serum, while a prior study had shown good results using CSF metabolomics[40]. The rate of advanced HE differed between the two cohorts, which could have affected the outcomes. Since thyroxine could be readily performed in the clinical laboratories, we focused only on it although it is possible that prediction using all 4 discovery-cohort biomarkers could yield better predictions. The validation cohort is relatively small but similar results were seen with respect to prediction of advanced HE; larger cohorts may be needed in future studies.

We conclude that in a large multi-center prospective inpatient cirrhosis cohort, admission serum metabolites associated with gut microbiota and low serum thyroxine predicts the future development of advanced HE independent of clinical biomarkers. The pattern of low serum thyroxine in patients who developed advanced HE was also found in a separate small prospective cohort using a clinical laboratory. Therefore, the use of serum metabolites, especially thyroxine, could provide useful prognostication on admission to the hospital and ensure rapid interventions to prevent the development of advanced hepatic encephalopathy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Transparency Statement:

Data is not available due to IRB restrictions

Abbreviations:

HE	hepatic encephalopathy
RFA	random forest analysis
BF	brain failure
AUC	area under the curve
ROC	receiver operating characteristic

ICU	intensive care unit
NACSELD	North American Consortium for the Study of End-Stage Liver Disease
ACLF	acute on chronic liver failure
FT4	free thyroxine

References:

1. Vilstrup H, Amodio P, Bajaj J, et al. Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. Hepatology 2014;60:715–35. [PubMed: 25042402]
2. Bajaj JS, O'Leary JG, Tandon P, et al. Targets to improve quality of care for patients with hepatic encephalopathy: data from a multi-centre cohort. Aliment Pharmacol Ther 2019;49:1518–1527. [PubMed: 31032966]
3. Cordoba J, Ventura-Cots M, Simon-Talero M, et al. Characteristics, risk factors, and mortality of cirrhotic patients hospitalized for hepatic encephalopathy with and without acute-on-chronic liver failure (ACLF). J Hepatol 2014;60:275–81. [PubMed: 24128414]
4. Hernaez R, Sola E, Moreau R, et al. Acute-on-chronic liver failure: an update. Gut 2017;66:541–553. [PubMed: 28053053]
5. Bajaj JS, O'Leary JG, Tandon P, et al. Hepatic Encephalopathy Is Associated With Mortality in Patients With Cirrhosis Independent of Other Extrahepatic Organ Failures. Clin Gastroenterol Hepatol 2017;15:565–574 e4. [PubMed: 27720916]
6. Pelle J, Castelli FA, Rudler M, et al. Metabolomics in the understanding and management of hepatic encephalopathy. Anal Biochem 2021:114477. [PubMed: 34808106]
7. McPhail MJ, Shawcross DL, Lewis MR, et al. Multivariate metabotyping of plasma predicts survival in patients with decompensated cirrhosis. J Hepatol 2016;64:1058–67. [PubMed: 26795831]
8. Bajaj JS, Fan S, Thacker LR, et al. Serum and urinary metabolomics and outcomes in cirrhosis. PLoS One 2019;14:e0223061. [PubMed: 31560724]
9. Ghabril M, Jackson M, Gotur R, et al. Most Individuals With Advanced Cirrhosis Have Sleep Disturbances, Which Are Associated With Poor Quality of Life. Clin Gastroenterol Hepatol 2017;15:1271–1278 e6. [PubMed: 28167158]
10. Mindikoglu AL, Opekun AR, Putluri N, et al. Unique metabolomic signature associated with hepatorenal dysfunction and mortality in cirrhosis. Transl Res 2018;195:25–47. [PubMed: 29291380]
11. Bajaj JS, Garcia-Tsao G, Reddy KR, et al. Admission urinary and serum metabolites predict renal outcomes in hospitalized patients with cirrhosis. Hepatology 2021.
12. O'Leary JG, Reddy KR, Garcia-Tsao G, et al. NACSELD acute-on-chronic liver failure (NACSELD-ACLF) score predicts 30-day survival in hospitalized patients with cirrhosis. Hepatology 2018;67:2367–2374. [PubMed: 29315693]
13. Bajaj JS, Reddy KR, O'Leary JG, et al. Serum Levels of Metabolites Produced by Intestinal Microbes and Lipid Moieties Independently Associated With Acute on Chronic Liver Failure and Death in Patients With Cirrhosis. Gastroenterology 2020.
14. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–845. [PubMed: 3203132]
15. Tranah TH, Vijay GK, Ryan JM, et al. Systemic inflammation and ammonia in hepatic encephalopathy. Metab Brain Dis 2013;28:1–5. [PubMed: 23224356]
16. Bajaj JS, Vargas HE, Reddy KR, et al. Association Between Intestinal Microbiota Collected at Hospital Admission and Outcomes of Patients With Cirrhosis. Clin Gastroenterol Hepatol 2019;17:756–765 e3. [PubMed: 30036646]

17. Hatton G, Shawcross DL. Is treating the gut microbiome the key to achieving better outcomes in cirrhosis? Expert Rev Gastroenterol Hepatol 2019;13:1–2. [PubMed: 30791837]
18. Beyoglu D, Idle JR. The metabolomic window into hepatobiliary disease. J Hepatol 2013;59:842–58. [PubMed: 23714158]
19. Wang J, Shen X, Jain R, et al. Establishing a novel biosynthetic pathway for the production of 3,4-dihydroxybutyric acid from xylose in Escherichia coli. Metab Eng 2017;41:39–45. [PubMed: 28342964]
20. Shinka T, Inoue Y, Ohse M, et al. Rapid and sensitive detection of urinary 4-hydroxybutyric acid and its related compounds by gas chromatography-mass spectrometry in a patient with succinic semialdehyde dehydrogenase deficiency. J Chromatogr B Analyt Technol Biomed Life Sci 2002;776:57–63.
21. Mousavi M, Jonsson P, Antti H, et al. Serum metabolomic biomarkers of dementia. Dement Geriatr Cogn Dis Extra 2014;4:252–62. [PubMed: 25177334]
22. Gamba P, Staurenghi E, Testa G, et al. A Crosstalk Between Brain Cholesterol Oxidation and Glucose Metabolism in Alzheimer's Disease. Front Neurosci 2019;13:556. [PubMed: 31213973]
23. Saeed A, Floris F, Andersson U, et al. 7alpha-hydroxy-3-oxo-4-cholestenoic acid in cerebrospinal fluid reflects the integrity of the blood-brain barrier. J Lipid Res 2014;55:313–8. [PubMed: 24319290]
24. Virdee MS, Saini N, Kay CD, et al. An enriched biosignature of gut microbiota-dependent metabolites characterizes maternal plasma in a mouse model of fetal alcohol spectrum disorder. Sci Rep 2021;11:248. [PubMed: 33420159]
25. Roager HM, Licht TR. Microbial tryptophan catabolites in health and disease. Nat Commun 2018;9:3294. [PubMed: 30120222]
26. Caussy C, Hsu C, Lo MT, et al. Link between gut-microbiome derived metabolite and shared gene-effects with hepatic steatosis and fibrosis in NAFLD. Hepatology 2018;68:918–932. [PubMed: 29572891]
27. Moreau R, Claria J, Aguilar F, et al. Blood metabolomics uncovers inflammation-associated mitochondrial dysfunction as a potential mechanism underlying ACLF. J Hepatol 2020;72:688–701. [PubMed: 31778751]
28. Claria J, Moreau R, Fenaille F, et al. Orchestration of Tryptophan-Kynurenine Pathway, Acute Decompensation, and Acute-on-Chronic Liver Failure in Cirrhosis. Hepatology 2019;69:1686–1701. [PubMed: 30521097]
29. Marchesini G, Fabbri A, Bianchi GP, et al. Hepatic conversion of amino nitrogen to urea nitrogen in hypothyroid patients and upon L-thyroxine therapy. Metabolism 1993;42:1263–9. [PubMed: 8412738]
30. Malik R, Hodgson H. The relationship between the thyroid gland and the liver. QJM 2002;95:559–69. [PubMed: 12205333]
31. Thobe N, Pilger P, Jones MP. Primary hypothyroidism masquerading as hepatic encephalopathy: case report and review of the literature. Postgrad Med J 2000;76:424–6. [PubMed: 10878207]
32. Rimar D, Kruzel-Davila E, Dori G, et al. Hyperammonemic coma--barking up the wrong tree. J Gen Intern Med 2007;22:549–52. [PubMed: 17372808]
33. Bajaj JS, Reddy KR, OLeary JG, et al. Serum Levels of Metabolites Produced by Intestinal Microbes and Lipid Moieties Independently Associated With Acute-on-Chronic Liver Failure and Death in Patients With Cirrhosis. Gastroenterology 2020;159:1715–1730 e12. [PubMed: 32687928]
34. Knezevic J, Starchl C, Tmava Berisha A, et al. Thyroid-Gut-Axis: How Does the Microbiota Influence Thyroid Function? Nutrients 2020;12.
35. Rahimi RS, Safadi R, Thabut D, et al. Efficacy and Safety of Ornithine Phenylacetate for Treating Overt Hepatic Encephalopathy in a Randomized Trial. Clin Gastroenterol Hepatol 2020.
36. Shawcross DL, Sharifi Y, Canavan JB, et al. Infection and systemic inflammation, not ammonia, are associated with Grade 3/4 hepatic encephalopathy, but not mortality in cirrhosis. J Hepatol 2011;54:640–9. [PubMed: 21163546]

37. Trebicka J, Amoros A, Pitarch C, et al. Addressing Profiles of Systemic Inflammation Across the Different Clinical Phenotypes of Acutely Decompensated Cirrhosis. Front Immunol 2019;10:476. [PubMed: 30941129]

38. Patidar KR, Thacker LR, Wade JB, et al. Covert hepatic encephalopathy is independently associated with poor survival and increased risk of hospitalization. Am J Gastroenterol 2014;109:1757–63. [PubMed: 25178701]

39. Praktiknjo M, Simon-Talero M, Romer J, et al. Total area of spontaneous portosystemic shunts independently predicts hepatic encephalopathy and mortality in liver cirrhosis. J Hepatol 2020;72:1140–1150. [PubMed: 31954206]

40. Weiss N, Barbier Saint Hilaire P, Colsch B, et al. Cerebrospinal fluid metabolomics highlights dysregulation of energy metabolism in overt hepatic encephalopathy. J Hepatol 2016;65:1120–1130. [PubMed: 27520878]

What you need to know

Background

- Advanced or grade 3–4 hepatic encephalopathy (HE) using the West-Haven criteria, is an important component of acute-on-chronic liver failure (ACLF) associated with high mortality and morbidity due to falls and aspiration pneumonia, but clinical predictors are suboptimal
- Metabolomics have been used to predict ACLF, death and renal outcomes but their impact on advanced HE is unclear
- It is also difficult to translate platform-based metabolomics into clinical practice due to time, cost, and turn-around issues.

Findings

- In a multi-center cohort of 602 inpatients with cirrhosis, serum metabolites drawn on admission showed differences in microbially-generated metabolites, thyroxine, phospholipids, isoleucine, and maltose in the 24% who developed advanced HE versus the rest.
- Four metabolites (low thyroxine and maltose and higher methyl-4-hydroxybenzoate sulfate and 3–4 dihydroxybutyrate) significantly improved the prediction of advanced HE when added to the clinical model; thyroxine alone was also significant in improving prediction.
- This low thyroxine as a predictor of advanced HE was validated in a separate inpatient cirrhosis cohort using a clinical laboratory.

Implications for Patient care

- These findings of low admission serum thyroxine levels detected in the local clinical laboratory could help select patients at highest risk for development of advanced HE and offering second-line therapies, airway protection and enhanced monitoring could improve outcomes

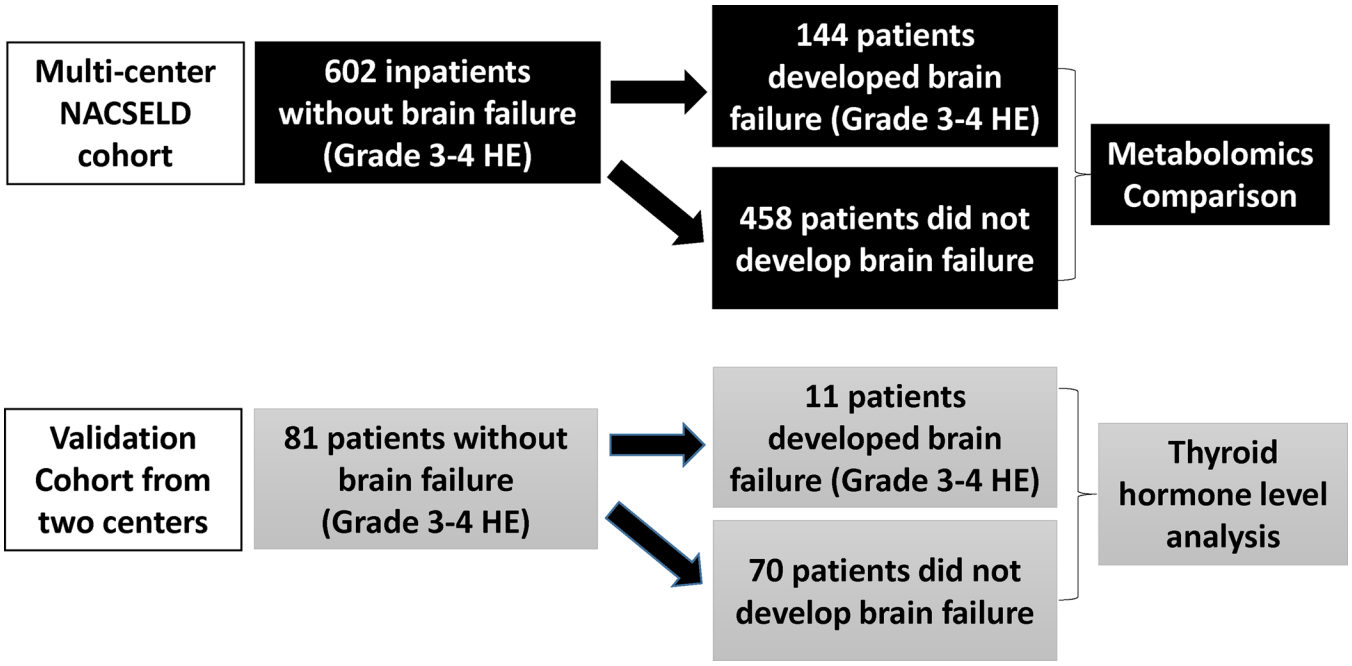

Figure 1: Schema of Study
Schema of the two cohorts

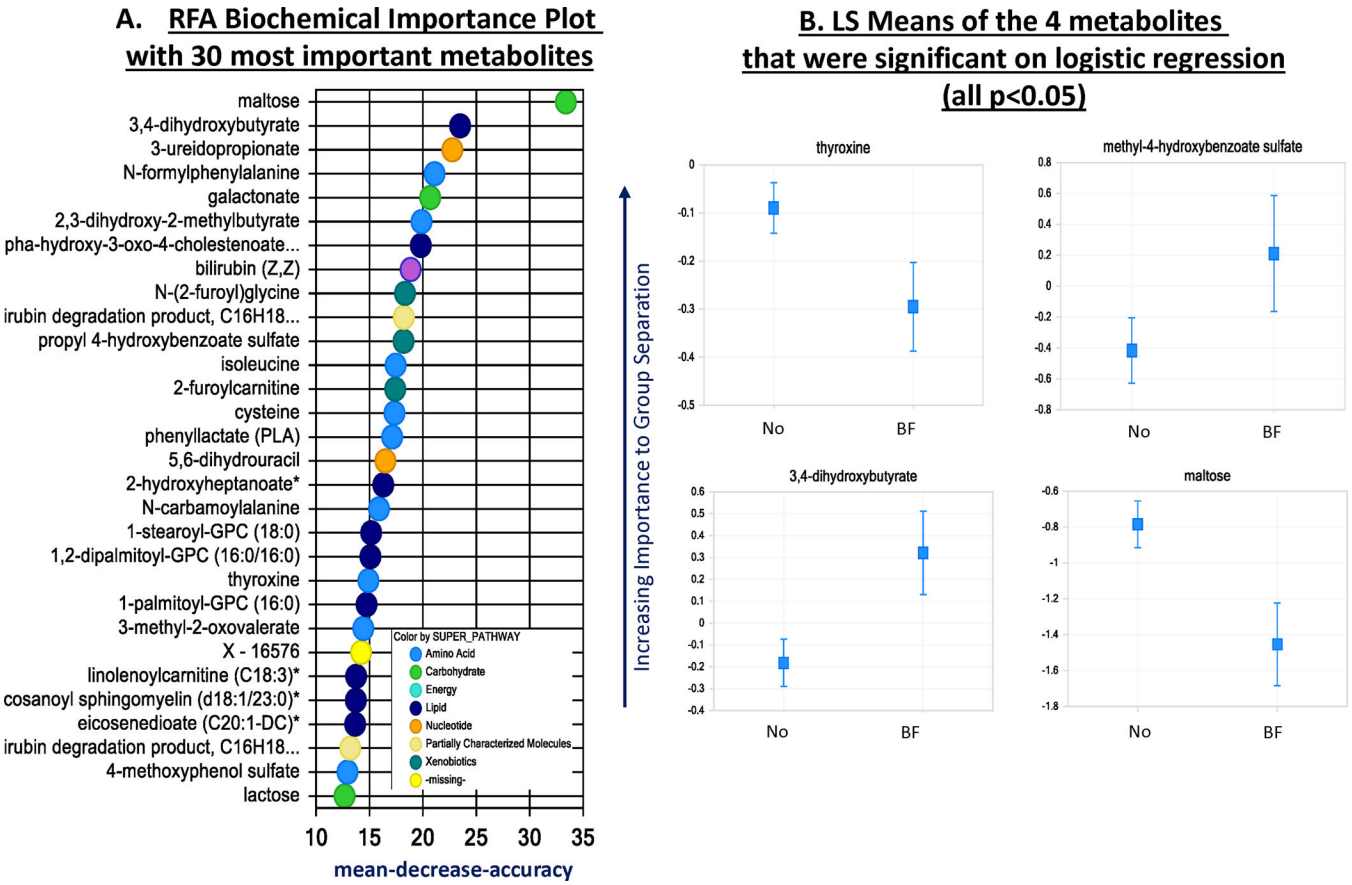

Figure 2: Random Forest Analysis and Least Squares Means of Metabolites

Random Forest Analysis and Least square means

2A: Biochemical importance plot of RFA showing the top 30 metabolites with the highest mean decrease accuracy (MDA) color-coded by pathways.

2B: Least-squares means (LS Means) of the four metabolites that were significant on logistic regression independent of clinical biomarkers (all $p<0.05$) with advanced HE or Brain failure (BF, brain failure) and those who did not (No)

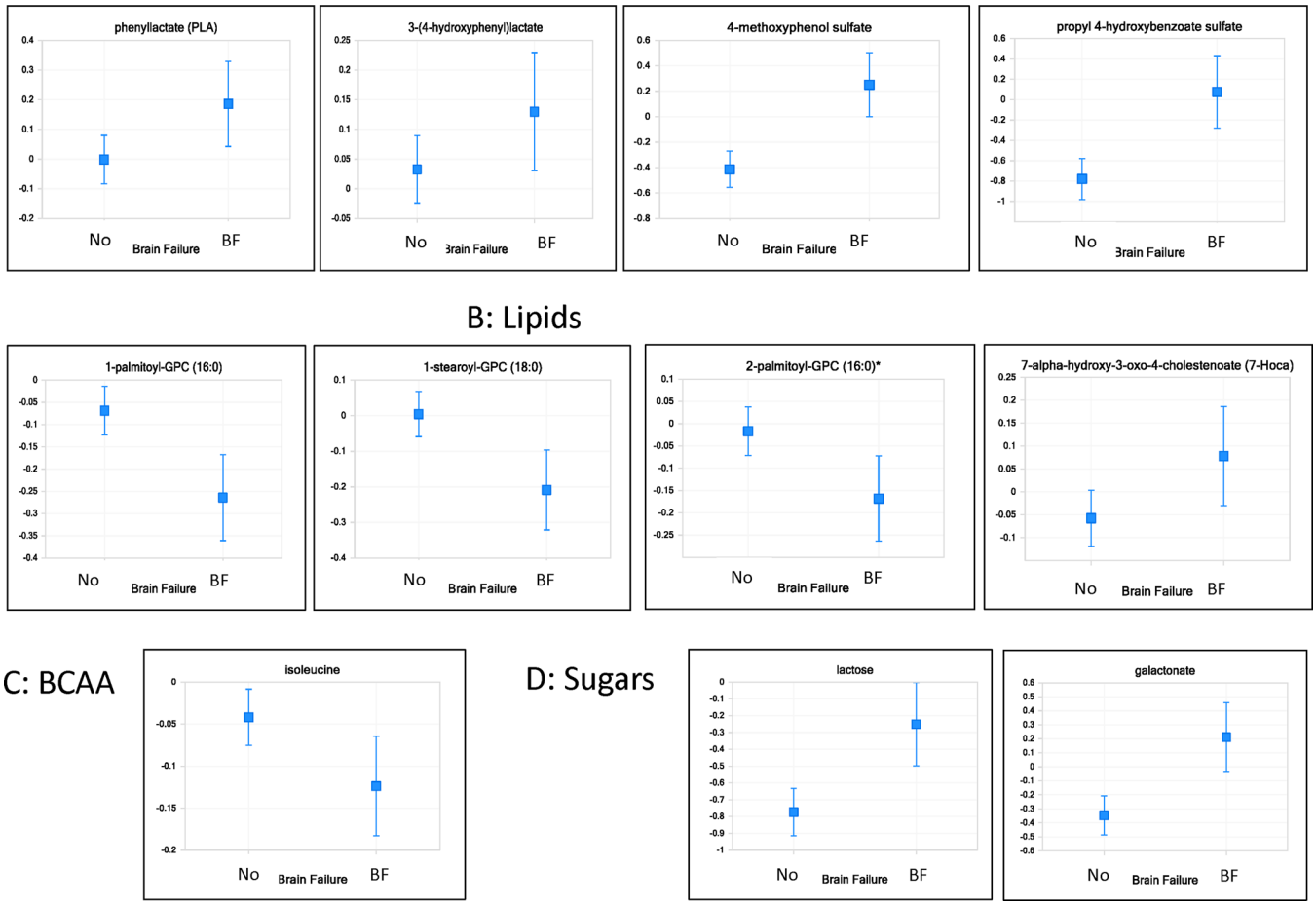

Figure 3: Least Squares means comparisons of other significant metabolites all p<0.05

Metabolite LS Means significantly different between those who developed advanced HE or BF (brain failure) versus not (No) (all p<0.05), excluding four metabolites already in figure 2

3A: Potential microbially-derived metabolites

3B: Lipids

3C: Branched chain amino acids (BCAA)

3D: Sugars

Table 1:

Clinical comparisons and outcomes between those with did and did not develop brain failure

	Did not Develop Advanced HE (n=458)	Developed Advanced HE (n=144)	P Value
Age (years)	56.3±10.0	55.3±8.1	0.25
Men (%)	279 (61%)	82 (57%)	0.40
Etiology (Alcohol, HCV, HCV+ alcohol, NASH, others)	128/115/70/84/61	49/33/16/22/24	0.36
Admission MELD score	18.6±7.5	22.0±7.8	<0.0001
Admission Na (mmol/L)	134.2±5.8	132.0±6.7	0.001
Admission WBC (/10^3/ml	7.9±5.0	8.2±4.8	0.49
Admission Albumin (g/dl)	2.85±0.7	2.68±0.68	0.01
Prior TIPS placement	50 (11%)	20 (14%)	0.33
Reason for admission *			
-Infections	151 (33%)	82 (57%)	<0.0001
-GI bleeding	73 (16%)	35 (24%)	0.012
-HE without infection	55 (12%)	52 (36%)	<0.0001
-Acute kidney injury	55 (12%)	27 (19%)	0.10
-Electrolyte changes	18 (4%)	5 (4%)	0.59
-Anasarca	59 (13%)	6 (4%)	0.001
-Liver-unrelated	82 (18%)	14 (10%)	0.08
Admission Rifaximin	140 (31%)	51 (35%)	0.03
Admission SBP prophylaxis	42 (9%)	14 (10%)	0.53
Hospital course			
Developed NACSELD-ACLF	34 (7%)	54 (38%)	<0.0001
Developed Respiratory failure	37 (8%)	35 (24%)	<0.0001
Developed Circulatory failure	40 (9%)	37 (26%)	<0.0001
Developed Renal failure	45 (10%)	36 (25%)	<0.0001
Developed AKI	169 (37%)	79 (55%)	<0.0001
Needed ICU transfer	86 (19%)	52 (36%)	<0.0001
Length of stay (days)	10.8±16.6	14.2±16.2	0.035
Inpatient death	9 (2%)	31 (22%)	<0.0001

* adds to more than the total due to >1 causes of admission listed.

Table 2:

Metabolites and direction on Random Forest Analysis between those with did and did not develop advanced HE

	Did not Develop advanced HE (n=458)	Developed advanced HE (n=144)
Aromatic amino acid metabolites		↑3-(4-hydroxyphenyl)lactate * ↑ phenyllactate * ↑ N-formylphenylalanine ↑4-methoxyphenol sulfate * ↓ **thyroxine**
Branched chain amino acid metabolites	↑Isoleucine	↑2,3 dihydroxy-2-methyl butyrate
Benzoate metabolism		↑Propyl-4-hydroxybenzoate sulfate * ↑**methyl-4-hydroxybenzoate sulfate** *
Short-chain fatty acid metabolites		↑**3–4 dihydroxybutyrate**
Carbohydrates	↑ **maltose**	↑ lactose, ↑galactonate
Lipids	↑1-Stearoyl-GPC ↑1-Palmitoyl-GPC ↑2-Palmitoyl-GPC	↑ 7alpha-Hydroxy-3-oxo-4-cholestenoate

* microbially-derived metabolites, Bold text: four metabolites that were also significantly additive to the basic clinical model on logistic regression

Table 3:

Characteristics of the validation cohort with respect to advanced HE

	Developed advanced HE		P value
	No (n=70)	Yes (n=11)	
Age (years)	54.5±9.3	51.8±13.4	0.542
Admission MELD-Na	20.9±8.9	22.0±7.9	0.53
Prior HE	33 (47%)	8 (73%)	0.11
Prior TIPS placement	5 (7%)	0 (0%)	1.0
Admission Lactulose	33 (47%)	8 (73%)	0.11
Admission Rifaximin	21 (30%)	6 (55%)	0.12
Admission Beta-blocker use	31 (44%)	3 (27%)	0.28
Admission SBP prophylaxis	7 (10%)	0 (0%)	0.58
Admission Serum albumin (g/dl)	2.8±0.6	2.99±0.52	0.37
Admission WBC count (/mm3)	7.9±4.3	8.2±4.6	0.81
Infections on admission	21 (30%)	5 (45%)	0.32
Grade 1–2 HE on admission	10 (14%)	6 (55%)	0.005
Admission Total thyroxine (ug/dL)	8.35±3.03	5.85±3.03	0.008
Admission Free T4 (uIU/mL)	1.13±0.23	0.92±0.26	0.026
Admission Thyroid uptake (%)	36.34±4.41	39.27±2.80	0.009
Admission TSH (ng/dl)	2.55±3.51	1.96±1.39	0.33

HE: hepatic encephalopathy, Data presented as mean±SD or in raw numbers (%). Comparisons performed using unpaired t-tests, Chi-square, or Fisher's exact test as appropriate