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Association of serum metabolites and gut microbiota at hospital admission with nosocomial infection development in patients with cirrhosis

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Abstract

Cirrhosis is complicated by a high rate of nosocomial infections (NIs), which result in poor outcomes and are challenging to predict using clinical variables alone. Our aim was to determine predictors of NI using admission serum metabolomics and gut microbiota in inpatients with

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

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cirrhosis. In this multicenter inpatient cirrhosis study, serum was collected on admission for liquid chromatography–mass spectrometry metabolomics, and a subset provided stool for 16SrRNA analysis. Hospital course, including NI development and death, were analyzed. Metabolomic analysis using analysis of covariance (ANCOVA) (demographics, Model for End-Stage Liver Disease [MELD] admission score, white blood count [WBC], rifaximin, and infection status adjusted) and random forest analyses for NI development were performed. Additional values of serum metabolites over clinical variables toward NI were evaluated using logistic regression. Stool microbiota and metabolomic correlations were compared in patients with and without NI development. A total of 602 patients (231 infection admissions) were included; 101 (17%) developed NIs, which resulted in worse inpatient outcomes, including intensive care unit transfer, organ failure, and death. A total of 127 patients also gave stool samples, and 20 of these patients developed NIs. The most common NIs were spontaneous bacterial peritonitis followed by urinary tract infection, *Clostridioides difficile*, and pneumonia. A total of 247 metabolites were significantly altered on ANCOVA. Higher MELD scores (odds ratio, 1.05; $p < 0.0001$), admission infection (odds ratio, 3.54; $p < 0.0001$), and admission WBC (odds ratio, 1.05; $p = 0.04$) predicted NI (area under the curve, 0.74), which increased to 0.77 ($p = 0.05$) with lower 1-linolenoyl-glycerolphosphoch oline (GPC) and 1-stearoyl-GPC and higher N-acetyltryptophan and N-acetyl isoputreanine. Commensal microbiota were lower and pathobionts were higher in those who developed NIs. Microbial–metabolite correlation networks were complex and dense in patients with NIs, especially sub-networks centered on Ruminococcaceae and Pseudomonadaceae. NIs are common and associated with poor outcomes in cirrhosis. Admission gut microbiota in patients with NIs showed higher pathobionts and lower commensal microbiota. Microbial–metabolomic correlations were more complex, dense, and homogeneous among those who developed NIs, indicating greater linkage strength. Serum metabolites and gut microbiota on admission are associated with NI development in cirrhosis.

INTRODUCTION

Patients with cirrhosis admitted with infections have a higher rate of organ failures, acute-on-chronic liver failure (ACLF), and death.[1] An all too frequent precursor for these negative outcomes is the development of a nosocomial infection (NI).[2] However, the predictive capability of admission clinical criteria for NI occurrence is suboptimal.[3] Moreover, some NIs are potentially preventable, which increases the need to adequately stratify the risk in hospitalized patients with cirrhosis.[4] A major contributor to poor outcomes in cirrhosis is the altered systemic inflammatory milieu and metabolome, part of which can be attributed to gut microbial change.[5] These alterations can contribute toward nosocomial and antibiotic-resistant infection development.[6,7] Prior studies have shown that gut microbial composition modulates cirrhosis progression, is already altered on admission in those who will develop ACLF, renal failure, or death and is different between those who have reached these outcomes versus the rest.[8–10] However, the role of microbial interaction with the host through metabolites is unclear.[11] We hypothesized that serum metabolites in patients with cirrhosis modulate the risk of development of NI.

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PATIENTS AND METHODS

We enrolled hospitalized patients with cirrhosis from 11 centers in the North American Consortium for the Study of End-Stage Liver Disease (NACSELD) between 2014 to 2017 who gave samples after informed consent. Patients with cirrhosis in the NACSELD included those nonelectively hospitalized, those without HIV infection, and patients with a prior transplant. Cirrhosis was diagnosed using liver biopsy, imaging, or endoscopic characteristics of varices in patients with chronic liver disease or those with evidence of decompensation. Serum was collected within 12 h of admission and a subset also provided stool samples before antibiotic therapy was instituted. Data were entered in the Research Electronic Data Capture database. Before study initiation, uniform sample collection practices were ensured at all sites. Samples were stored in −80°C freezers until analysis. Data pertaining to demographics, cirrhosis details, medications, reasons for admission, laboratory results, and hospital course were recorded as well as infections on admission and NI development. All infections were diagnosed per definitions used in prior publications and Infectious Diseases Society of America guidelines.[3]

Analyses were performed at Metabolon Inc. (Morrisville, NC) using validated ultrahigh performance liquid chromatography–tandem mass spectroscopy.[12] Analysis of covariance (ANCOVA) adjusting for age, sex, alcohol-associated etiology, Model for End-Stage Liver Disease (MELD) admission score, white blood count (WBC), infection, serum sodium, and serum albumin using false discovery rate (FDR) adjustment, represented by the q value, were performed to account for variability related to patient-level variables. After log transformation and imputation of missing values, if any, with the minimum observed value for each compound, analysis of variance contrasts and Welch's two-sample t tests were used to determine metabolites that were different between groups. Then an ANCOVA was performed. An estimate of the FDR was calculated to take into account the multiple comparisons that normally occur in metabolomic-based studies.[7] Instrument variability was determined by calculating the median relative standard deviation (RSD) for the internal standards that were added to each sample prior to injection into the mass spectrometers. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., noninstrument standards) present in 100% of the client matrix samples, which are technical replicates of pooled client samples. Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., noninstrument standards) present in the technical replicates.

Metabolites that were independently associated with the development of NI by ANCOVA were considered predictive of such outcomes. The ANCOVA tables were ranked according to p values, FDRs, and pathways found to be consistently involved in protection from or association with the outcomes and then were explored deeper for each outcome. Random forest analysis (RFA) was performed next and is a supervised classification technique based on an ensemble of decision trees.[13] To determine which metabolites make the largest contribution to the classification, a "variable importance" measure called the "mean decrease accuracy" (MDA) was computed. The MDA is determined by randomly permuting a variable, running the observed values through the trees, and then reassessing the prediction accuracy. If a variable is not important, then this procedure will result in little change

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in the accuracy of the class prediction (permuting random noise will give random noise). By contrast, if a variable is important to the classification, the prediction accuracy will drop after such a permutation, which we record as the MDA. Thus, the RFAs provide an "importance" rank ordering of metabolites, and the first 30 for each outcome are displayed. Areas under the curve (AUCs) for all metabolites were calculated for the ANCOVA-adjusted models for each category, including those with/without admission infection. Finally, logistic regression models to predict NI development were created from admission clinical variables only (age, admission values of WBC, serum sodium, serum albumin, MELD-sodium score, and admission infection) and then clinical models plus metabolites significant on RFA. From these models, receiver operator characteristic (ROC) curves were calculated, and the AUCs with 95% confidence intervals (CIs) were calculated. Finally, the AUC values for the clinical variables only and combined models were compared using the nonparametric method of DeLong et al.[14] for ≥2 correlated ROC curves.

Microbiome analysis

Microbial DNA was extracted from stool using published techniques, and 16SrRNA sequencing was performed.[15] Comparisons between patients who developed NI versus those who did not were performed using Linear discriminant analysis effect size (LEfSe).[16] Correlation networks of serum metabolites with microbiota composition were performed using published R (R Foundation for Statistical Computing) techniques.[17] Correlation linkages with $p < 0.05$ and $r > 0.6$ or <-0.6 were included and visualized in Cytoscape (Cytoscape Consortium, San Diego, CA, USA).

Regulatory

The study was approved by institutional review boards at all sites, and all patients provided informed consent before study procedures were undertaken.

RESULTS

Patient details

We included 602 patients who provided serum on admission. Of these, 376 were men and 38.4% ($n = 231$) had infections on admission. Of the 602 patients, 101 (17%) developed NIs at a median of 5 ± 2 days after admission. As shown in Table 1, NIs were associated with a higher rate of admission infection and higher MELD scores and WBCs. Patients who developed NIs had longer hospital lengths of stay and more frequently needed intensive care unit (ICU) transfer, experienced individual organ failures, developed ACLF, and died (Table 1). A total of 127 patients also provided an admission stool sample, and 20 of these patients later developed NIs (Figure 1).

Infection details

Of 602 patients, 237 had infections on admission, of which 170 (71.2%) did not develop any further infection. This contrasts with 331 of the 365 (90.7%) patients without an admission infection who remained NI free. Therefore, 501 patients remained NI free (Table 2). Of the 101 patients who developed NIs, 67 patients had one admission infection and then developed one or more additional infections after 48h (three patients developed two NIs and 64 patients

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developed one NI for a total of 70 NIs; Table S1). The 34 patients without an admission infection developed one or more NIs after 48h (three patients developed two NIs and 34 patients developed one NI for a total of 37 NIs). The most common initial admission infection was spontaneous bacterial peritonitis (SBP) followed by urinarytractinfection (UTI) and respiratory tract infections. All admission infections were bacterial in nature. NIs were more likely to be fungal ($n = 39$; $p < 0.0001$ vs. admission infections) with the remainder of NIs being caused by bacteria. The most common NIs were also SBP and UTIs but with higher rates of *Clostridioides difficile* and respiratory tract infections. Resistant organisms were more frequent causes of NI than admission infections: fluoroquinolone resistance, 14% versus 2% ($p < 0.0001$); vancomycin-resistant enterococci, 9% versus 3% ($p = 0.01$); and methicillin-resistant *Staphylococcus* aureus, 2% versus 5% ($p = 0.08$).

Metabolomics

We identified 1464 metabolites in total, of which 1196 were named. The majority were lipid-related metabolites ($n = 459$), then xenobiotics ($n = 329$) and amino acids ($n = 223$) followed by nucleotides ($n = 43$), peptides ($n = 40$), cofactors and vitamins ($n = 36$), partially characterized metabolites ($n = 27$), and carbohydrates ($n = 26$). A total of 247 metabolites were significantly increased using ANCOVA. Specific metabolite fold changes that differed between those who developed NI versus not on ANCOVA are shown in Tables S1 and S1. The top 25 metabolites on RFA are shown in Table 3 and according to the MDA in Figure 2.

Logistic regression

Higher MELD score (odds ratio [OR], 1.05; 95% CI, 1.02–1.09; $p < 0.0001$), admission infection (OR, 3.54; 95% CI, 2.18–5.76; $p < 0.0001$), and admission WBC (OR, 1.05; 95% CI, 1.00–1.09; $p = 0.04$) were significant predictors of NI development with an AUC of 0.74. Of the metabolites, a decrease in 1-linolenoyl-glycerolphosph ocholine (GPC) and 1-stearoyl-GPC and an increase in N-acetyl-tryptophan and N-acetyl isoputreanine added significantly to the model (AUC, 0.77; $p = 0.05$).

Microbiota analysis

On LEFse, Fusobacteriace and Pseudomonadaceae at the family level and *Fusobacterium*, *Hydrogenoaero-bacterium*, and *Ruminococcus* at the genus level were associated with a lower risk of NI development, whereas Gammaproteobacteria, Corynebacteriaceae, and Alteromonadales at the family level and *Corynebacterium*, *Hungatella*, and *Pluralibacter* at the genus level were associated with NI development (Figure 3).

Correlation network analysis

The analysis of patients who developed NIs was more centralized, dense, and complex than the analysis of patients without NIs. The NI correlation network had 1138 nodes with an average of 41.16 neighbors, a centralization of 0.13 with a density of 0.036, and a heterogeneity of 0.96, whereas the non-NI network had 956 nodes, an average of 21.47 neighbors, a density of 0.022, a heterogeneity of 1.33, and a centralization of 0.11 (Figure 4).

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We then focused on bacterial taxa from admission stool samples that are important in cirrhosis prognostication. We found in those who developed an NI, Ruminococcaceae had a complex correlation network with negative correlations with metabolites that were associated independently with NI (5-hydroxylysine, 3[4-hydroxyphenyl]propionate), pyruvate; Figure 5A. In those who did not develop an NI, stool Ruminococcaceae was negatively associated with stool Enterococcaceae (Figure 5B). Similarly with the family Lachnospiraceae, which has also been associated with short-chain fatty acid production, there was a negative linkage with metabolites associated with (N(1) = N(8))-acetylspermidine and N–acetyl-isoputreanine that were significantly linked with NI on RFA (Figure 5C). In addition, Lachnospiraceae in stool was negatively linked with other metabolites associated with NI (N,N,N-trimethyl-L-alanyl-L-proline betaine [TMAP], N-acetyl valine, asymmetric dimethyl arginine [ADMA] + symmetric dimethyl arginine [SDMA]) and positively linked with arachidoylcholine that was lower in those who developed NIs. In those without NI, stool Lachnospiraceae, similar to Ruminococcaceae, was negatively associated with Enterococcaceae (Figure 5D). Stool Pseudomonadaceae was negatively associated with choline, myo-inositol, and taurine and positively linked with bilirubin, bilirubin degradation products, and citrate (Figure 5E). No significant correlations were found with stool Pseudomonadaceae in the non-NI group.

DISCUSSION

Patients with cirrhosis are prone to NIs that are associated with poor outcomes.[18] However, NIs are difficult to predict and often only diagnosed after antibiotic failures or organ failures have occurred.[19] Prior studies using clinical criteria alone to predict NI development in hospitalized patients with cirrhosis have been relatively inaccurate.[3] Therefore, other biomarkers are needed. In a large, prospectively collected, nonelectively hospitalized cohort of patients with cirrhosis, we found that admission serum metabolites, focusing on choline, polyamine, and bacterial metabolites, were uniquely associated with NI prediction and could potentially add to clinical biomarkers. We also found significant associations between gut microbial pathobionts and lower commensals and NIs that were differentially associated with serum metabolites.

As expected from prior studies, patients who developed NIs compared with those who did not had worse clinical profiles at admission and poor outcomes during admission.[20] Most NIs were in patients already admitted with an infection, and several patients had more than one NI. In addition, NIs were more likely to be caused by resistant organisms and fungi compared with index community-acquired or health care–associated infections.[1] As a result, we need to prevent or at least detect NIs earlier, which necessitates the study and implementation of tailored interventions that may also serve to prevent ACLF and death. This is necessary because prior studies in larger cohorts, including this study, had poor predictive capability for NI development when restricted only to clinical variables.[3]

The specific admission serum metabolites associated with protection from subsequent NI development were choline metabolites, including 1-stearoyl and lineloylcholine. These metabolites are precursors of the cell membrane required for cell membrane stability and are also markers of hepatic regeneration.[7] Their relative decrease in serum has been

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associated with poor outcomes in prior studies of liver disease and cirrhosis.[7,21] Although lower levels of some of these metabolites were also associated with ACLF and death, their relative abundance may represent “healthier” liver function. In this study, high serum levels of these metabolites were associated with a lower NI development after controlling for clinical biomarkers such as MELD score and serum albumin. In contrast, the specific serum metabolites associated with NI development were N-acetylated amino acids, sex steroids and homologs, and dicarboxylic acids (suberate and pimelate). N-acetyltryptophan is a potential uremic toxin that could be associated with NI as an early marker of kidney dysfunction.[22] High levels of estrone and genistein, which are internal and external sources of female sex steroids, respectively, have been associated with poor prognosis in patients with cirrhosis in general.[12]

Although some of these metabolites were altered in patients in our cohort who went on to develop ACLF and died, there are some metabolites specific for NI. Choline and phosphocholine moieties, estrogenic metabolites, and N-acetyltryptophan and N-acetyl isoputreanine were associated with the major negative outcomes. However, N-acetyltryptophan, pimelate, lanthionine, 4-imidazoleacetate, and suberate were relatively specific for NI occurrence. N-acetylisoputreanine is a metabolite formed after spermidine is degraded and has been associated with kidney dysfunction and cognitive impairment.[23] Pimelate is important for the bacterial synthesis of biotin, a vitamin critical for fatty acid synthesis, branched-chain amino acid catabolism, and gluconeogenesis.[24] Lanthionine is an amino acid derivative important for bacterial cell wall and toxin synthesis and could be a marker of bacterial function and a uremic toxin.[25,26]

In addition to metabolites, gut microbiota can predict outcomes in cirrhosis, and in the relatively smaller subset who provided stool samples, there were higher commensals and lower pathobionts in patients who did not develop NI versus the rest. These were related to bacteria that produce short-chain fatty acids such as Ruminococcaceae and those that have pathogenic taxa such as Pseudomonadaceae.[27] However, the interaction between metabolites and bacteria could shed greater light into the pathogenesis of NIs as well as provide microbially based targets for the prevention of NIs in this population. This is important because several metabolites that differentiated NIs versus no NI are associated with bacterial metabolism as discussed previously. Specifically, these are related to the phylum Proteobacteria that are gram-negative rods responsible for a major share of infections in cirrhosis.[4] The overall bacterial–metabolite linkage was denser, more complex, and with a homogeneous network in those who developed NI compared with those who were protected, which could indicate a relatively closer relationship between bacteria and metabolites in this subset.

In those who developed NIs, stool commensals such as Ruminococcaceae and Lachnospiraceae were negatively linked with metabolites that were associated with NI development (5-hydroxylysine, 3[4-hydroxyphenyl] propionate, acetylspermidine, N-acetylisoputreanine, TMAP, N-acetyl valine, and ADMA+SDMA). These serum metabolites are related to vascular and kidney dysfunction and could predispose to a milieu related to immune dysfunction and end-organ damage.[28,29] On the other hand, pathobiont-containing

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families such as Pseudomonadaceae were negatively associated with serum levels of choline and myo-inositol, which usually indicate better liver health.[7]

The data here show that specific admission serum metabolites are over and above clinical biomarkers, and parameters could be developed as potential biomarkers for later NI development. These metabolites are distinct from metabolites that associate with other clinical complications, such as advanced hepatic encephalopathy (HE) and kidney failure, and there are several metabolites that are unique to NI that were analyzed in the same cohort.[12,30,31]

Although NIs vary in underlying microbiology and antibiotic use, we adjusted for these by using admission infection status and other clinical data available while ensuring that only baseline admission samples were analyzed. Therefore, although patients who developed NIs had higher antimicrobial metabolites in the serum, this was not significant in multivariable analysis. Despite our efforts, the AUCs for addition of serum metabolites to clinical information were modest but impactful on our understanding of the potential risk factors for NI development. We did not consider rectal swabs but used stools, which could have different results.[32] However, we focused on the entire microbiome rather than specific organisms for NI. The focus on gut microbial comparisons and linkages provides another target to prevent NI development and potentially ACLF and death.

In a large, prospective, multicenter cohort of non-electively admitted patients with cirrhosis, we found that a fifth of patients developed NIs. NIs were often caused by resistant bacteria or fungal organisms and resulted in a higher probability of ACLF and death. Specific admission serum metabolites significantly added to the predictive capability for development of NIs. These serum metabolites could serve as potential biomarkers to identify subgroups at greater risk for NIs, and the linkage with bacteria could increase our insight into the pathophysiology of NI development in cirrhosis.

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DATA AVAILABILITY STATEMENT

Because of institutional review board restrictions, individual patient-level clinical, metabolomic, or microbial data will not be made available to others.

Abbreviations:

ACLF	acute-on-chronic liver failure

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ADMA	asymmetric dimethyl arginine
AKI	acute kidney injury
ANCOVA	analysis of covariance
AUC	area under the curve
CI	confidence interval
FDR	false discovery rate
HCV	hepatitis C virus
HE	hepatic encephalopathy
ICU	intensive care unit
LDA	linear discriminant analysis
LEfSe	linear discriminant analysis effect size
MDA	mean decrease accuracy
MELD	Model for End-Stage Liver Disease
NACSELD	North American Consortium for the Study of End-Stage Liver Disease
NAFLD	nonalcoholic fatty liver disease
NI	nosocomial infection
OR	odds ratio
RFA	random forest analysis
ROC	receiver operator characteristic
RSD	relative standard deviation
SBP	spontaneous bacterial peritonitis
SDMA	symmetric dimethyl arginine
TMAP	N,N,N-trimethyl-l-alanyl-l-proline betaine
UTI	urinary tract infection
WBC	white blood count

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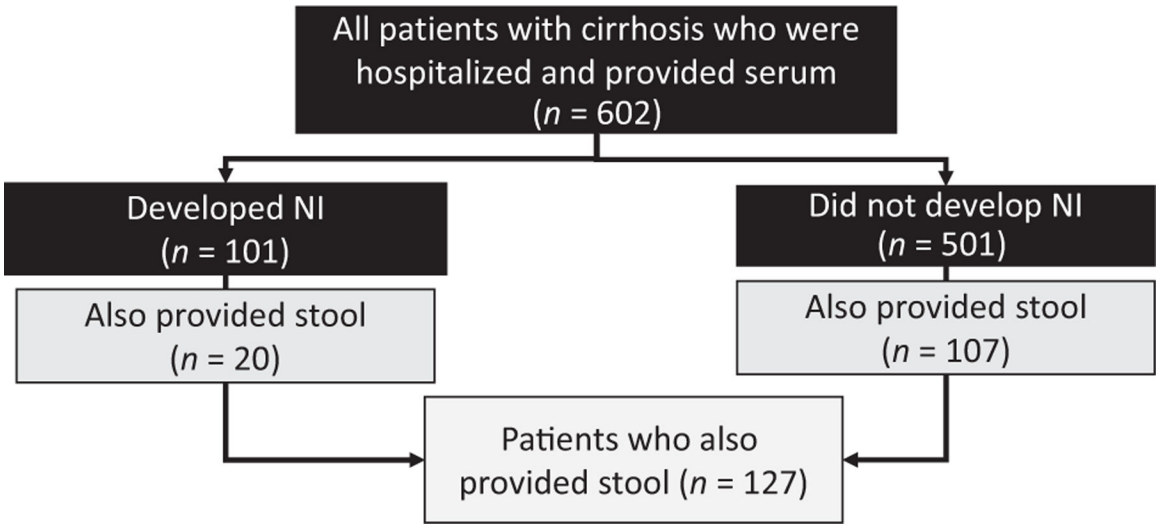

FIGURE 1.
Flowchart of patients.

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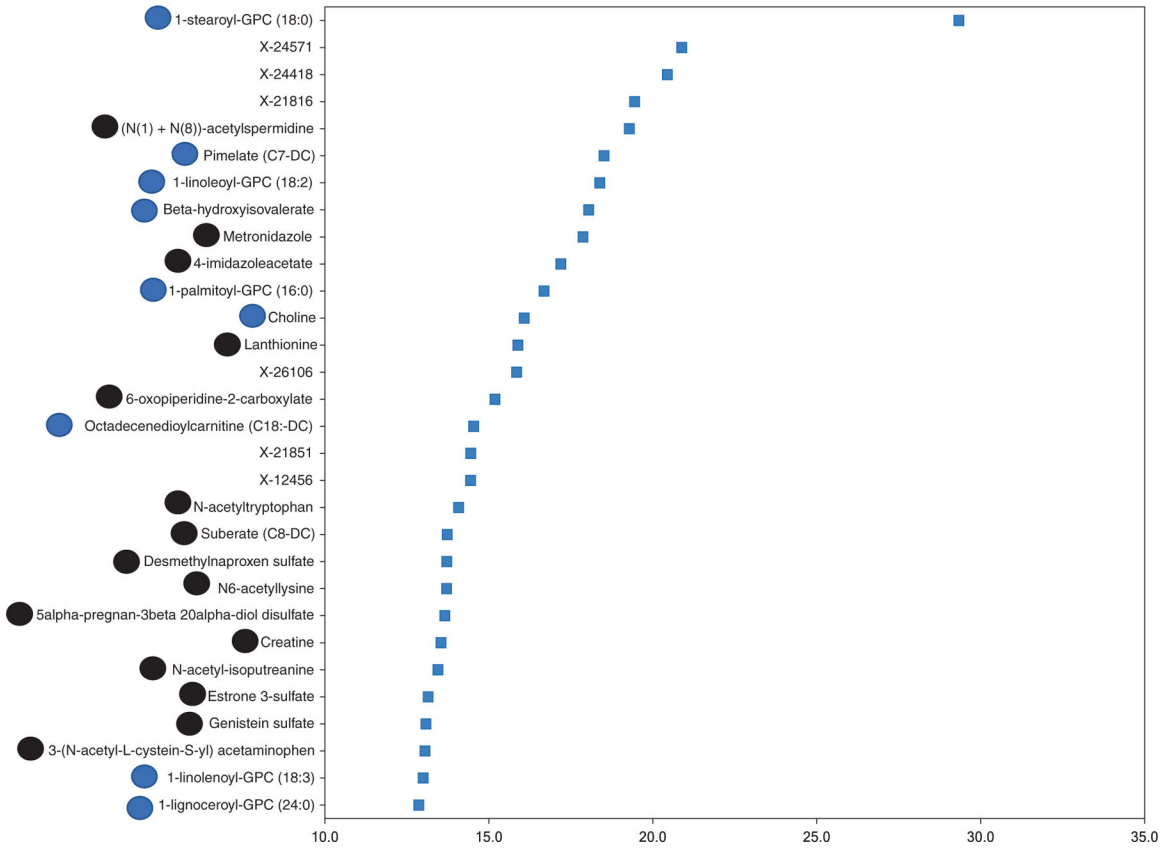

FIGURE 2.

RFA analysis for NIs. Black dots indicate measurements were higher in those who developed NIs, and blue dots indicate measurements were lower in those who developed NIs. Metabolites with “X-” indicate unknown metabolites.

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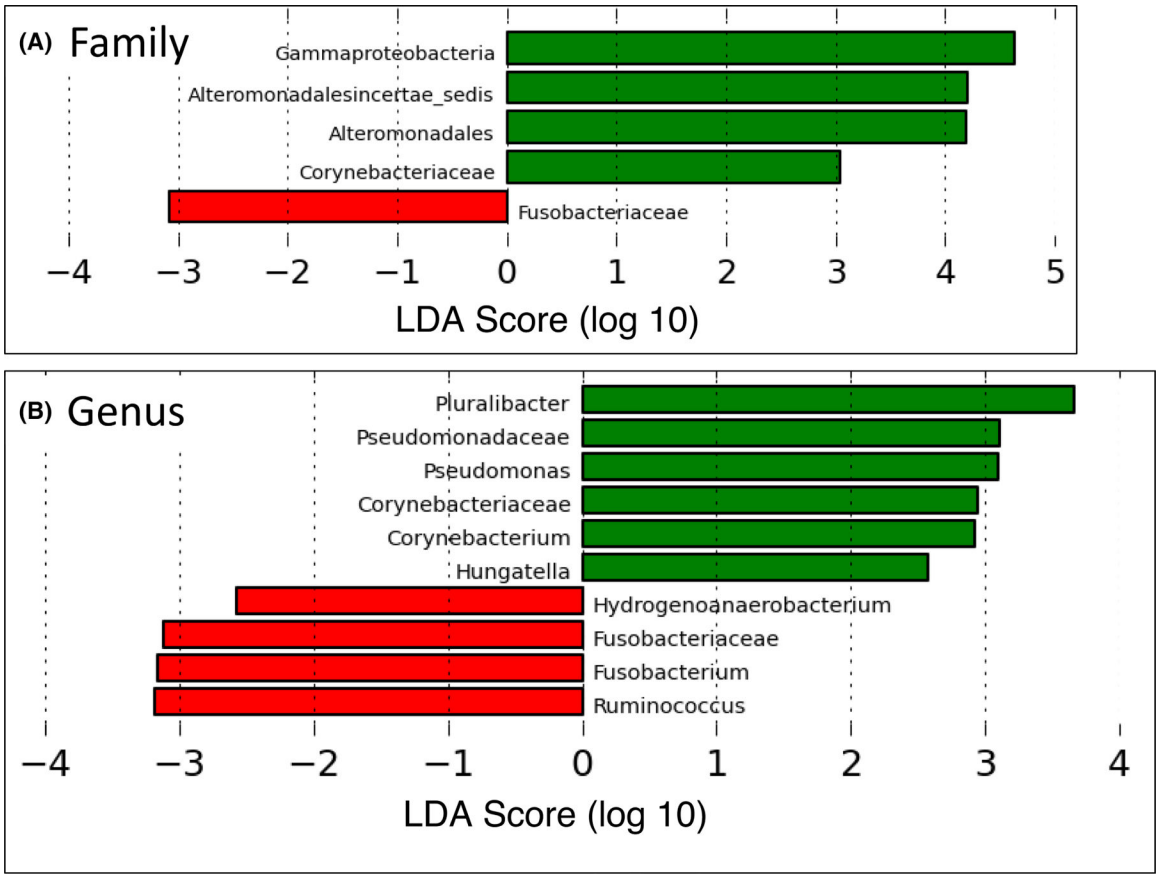

FIGURE 3.

Microbiota LEfSe on NIs. LEfSe with green indicates NIs developed, and red indicates NIs did not develop.

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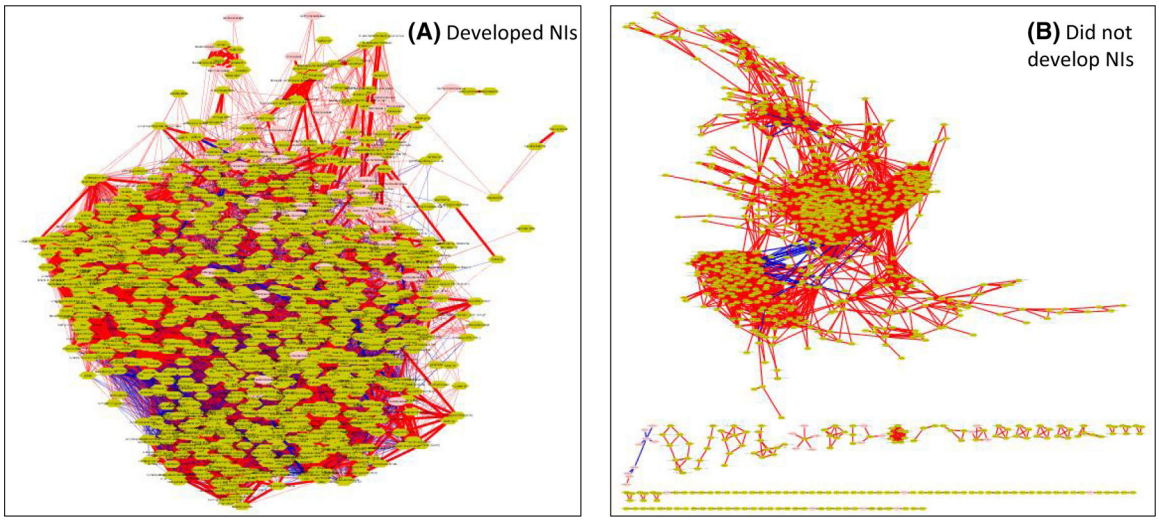

FIGURE 4.

Overall microbial–metabolite correlation network. Pink indicates bacteria, green indicates metabolites, red lines indicate positive linkage, and blue lines indicate negative linkage. (A) Those who developed NIs had a complex, dense, and homogeneous correlation network between microbiota and metabolites. (B) Those who did not develop NIs had a loose and heterogeneous correlation network between microbiota and metabolites.

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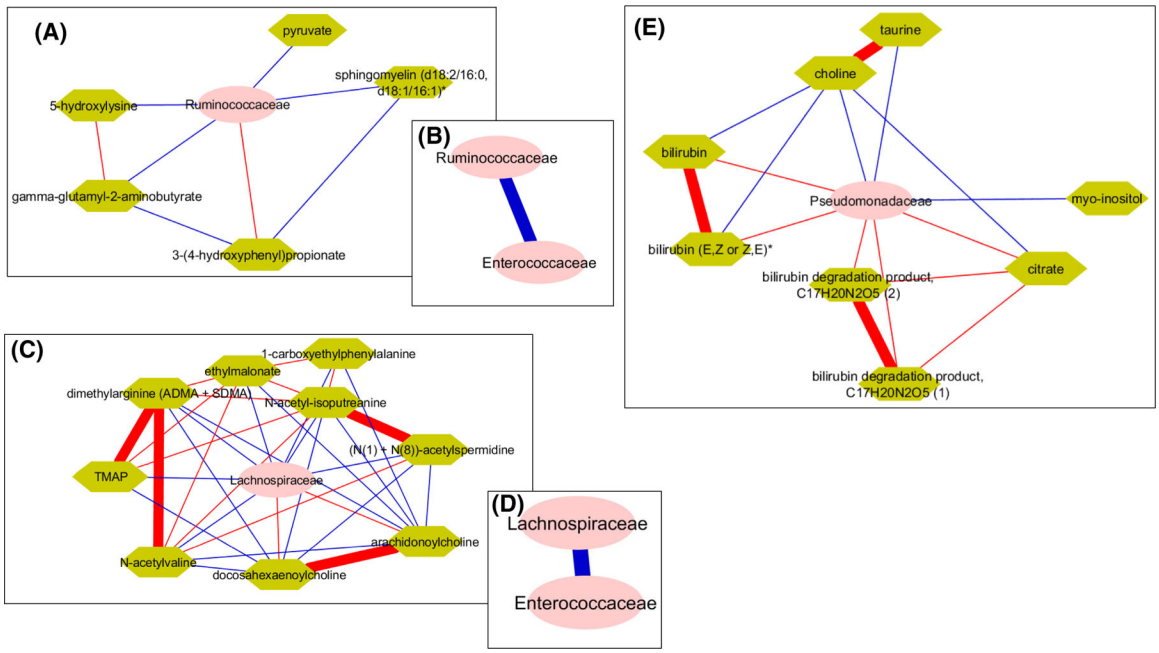

FIGURE 5.

Correlation network analysis subnetworks. Pink indicates bacteria, green indicates metabolites, red lines indicate positive linkage, and blue lines indicate negative linkage. (A) Ruminococcaceae subnetwork in those who developed NIs. (B) Ruminococcaceae subnetwork in those who did not develop NIs. (C) Lachnospiraceae subnetwork in those who developed NIs. (D) Lachnospiraceae subnetwork in those who did not develop NIs. (E) Pseudomonadaceae subnetwork in those who developed NIs.

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TABLE 1

Clinical presentation and course of patients who developed NI versus others

	Did not develop NI (*n* = 501) (SD)	**Developed NI (*n* = 101) (SD)**	***p* value**
Age, y	56.1 ± 9.5	55.6 ± 9.7	0.66
Sex, male, *n* (%)	307 (61)	59 (59)	0.43
Cirrhosis etiology, HCV/Alcohol/HCV + Alcohol/NAFLD/other, *n*	130/132/8zg0/86/73	21/28/11/19/22	0.15
Admission values			
Infections on day of admission, *n* (%)	170 (34)	67 (67)	<0.0001
On rifaximin, *n* (%)	211 (42)	44 (44)	0.71
On SBP prophylaxis, *n* (%)	60 (12)	10 (10)	0.56
MELD score	18.8 ± 7.5	22.8 ± 8.2	<0.0001
Serum sodium, Meq/L	134.1 ± 6.0	131.8 ± 6.0	<0.0001
Serum albumin, g/dl	2.83 ± 0.67	2.78 ± 0.65	0.55
WBC, $X10^3/mm^3$	7.56 ± 4.52	9.73 ± 6.28	0.001
Course			
Length of stay, days	8.3 ± 11.0	27.2 ± 26.7	<0.0001
AKI development, *n* (%)	150 (30)	71 (71)	<0.0001
ICU transfer, *n* (%)	83 (17)	61(61)	<0.0001
Requiring dialysis, *n* (%)	43 (9)	38 (38)	<0.0001
Developed Grade 3–4 HE, *n* (%)	96 (18)	41 (41)	<0.0001
Required pressors, *n* (%)	37 (7)	37 (37)	<0.0001
Required mechanical ventilation, *n* (%)	35 (7)	41 (41)	<0.0001
ACLF, *n* (%)	46 (9)	42 (42)	<0.0001
Inpatient death, *n* (%)	15 (3)	26 (26)	<0.0001
Hospice referral, *n* (%)	17 (3)	14 (14)	<0.0001

Abbreviations: ACLF, acute-on-chronic liver failure; AKI, acute kidney injury; HCV, hepatitis C virus; HE, hepatic encephalopathy; ICU, intensive care unit; MELD, Model for End-Stage Liver Disease; NAFLD, nonalcoholic fatty liver disease; NI, nosocomial infection; SBP, spontaneous bacterial peritonitis; WBC, white blood count.

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TABLE 2

Details of admission infection

	Did not develop NI (n = 501)[a]	Subsequently developed NI (n = 101)	p value
Infections on admission	170 (34)	67 (67)	<0.0001
Spontaneous bacterial peritonitis	50 (10)	13 (13)	0.40
Spontaneous bacteremia	21 (4)	10 (10)	0.01
Respiratory tract infection	12 (2)	3 (3)	0.73
Skin and soft tissue	21 (4)	9 (9)	0.05
Urinary tract infection	33 (6)	18 (18)	0.001
Clostridioides difficile infection	10 (2)	7 (7)	0.006
Intra-abdominal infection	7 (1)	4 (4)	0.09
Secondary bacterial peritonitis	1 (0.1)	0 (0)	1.0
Procedure related	0 (0)	1 (1)	1.0
Other infections	15 (3)	2 (2)	0.76
Resistance details			
Methicillin-resistant *Staphylococcus aureus*	2 (0.3)	2 (2)	1.0
Vancomycin-resistant enterococci	3 (0.6)	4 (4)	1.0
Fluoroquinolone resistance	3 (0.6)	2 (2)	1.0

Note: Data are provided as n (%).

Abbreviation: NI, nosocomial infection.

[a]Did not develop any further infections.

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TABLE 3

Named metabolites significant on random forest analysis

Lower in NIs	Higher in NIs
1-Stearoyl-GPC	Pimelate
1-Linoleoyl-GPC	4-Imidazoleacetate
1-Palmitoyl-GPC	Lanthionine
Choline	6-Oxoperidine-2-carboxylate
1-Linolenoyl-GPC	N-acetyltryptophan
1-Lignoceroyl-GPC	Suberate
Octadecenedioylcarnitine (C18:-DC)	5-Alpha-prenan-3beta-20 alpha diol sulfate
	N-acetyl isoputreanine
	Estrone-3-sulfate
	Genistein sulfate
	(N(1) + N(8))-acetylspermidine

Abbreviation: DC, dioylcarninite; GPC, glycerolphosphocholine; NI, nosocomial infection.