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Admission Urinary and Serum Metabolites Predict Renal Outcomes in Hospitalized Patients With Cirrhosis

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Abstract

BACKGROUND AND AIMS: Acute kidney injury (AKI) has a poor prognosis in cirrhosis. Given the variability of creatinine, the prediction of AKI and dialysis by other markers is needed. The aim of this study is to determine the role of serum and urine metabolomics in the prediction of AKI and dialysis in an inpatient cirrhosis cohort.

APPROACH AND RESULTS: Inpatients with cirrhosis from 11 North American Consortium of End-stage Liver Disease centers who provided admission serum/urine when they were AKI

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and dialysis-free were included. Analysis of covariance adjusted for demographics, infection, and cirrhosis severity was performed to identify metabolites that differed among patients (1) who developed AKI or not; (2) required dialysis or not; and/pr (3) within AKI subgroups who needed dialysis or not. We performed random forest and AUC analyses to identify specific metabolite(s) associated with outcomes. Logistic regression with clinical variables with/without metabolites was performed. A total of 602 patients gave serum (218 developed AKI, 80 needed dialysis) and 435 gave urine (164 developed AKI, 61 needed dialysis). For AKI prediction, clinical factor–adjusted AUC was 0.91 for serum and 0.88 for urine. Major metabolites such as uremic toxins (2,3-dihydroxy-5-methylthio-4-pentenoic acid [DMTPA], N2N2dimethylguanosine, uridine/pseudouridine) and tryptophan/tyrosine metabolites (kynunerate, 8-methoxykyunerate, quinolinate) were higher in patients who developed AKI. For dialysis prediction, clinical factor–adjusted AUC was 0.93 for serum and 0.91 for urine. Similar metabolites as AKI were altered here. For dialysis prediction in those with AKI, the AUC was 0.81 and 0.79 for serum/urine. Lower branched-chain amino-acid (BCAA) metabolites but higher cysteine, tryptophan, glutamate, and DMTPA were seen in patients with AKI needing dialysis. Serum/urine metabolites were additive to clinical variables for all outcomes.

CONCLUSIONS: Specific admission urinary and serum metabolites were significantly additive to clinical variables to predict AKI development and dialysis initiation in inpatients with cirrhosis. These observations can potentially facilitate earlier initiation of renoprotective measures.

Acute kidney injury (AKI) is one of the most common complications in hospitalized patients with cirrhosis, and the one associated with the highest mortality.[1,2] Its diagnosis is based on changes in serum creatinine (sCr).[3] Newer serum and urine biomarkers are being developed to aid in the differential diagnosis of AKI that will guide therapy[4]; however, trying to predict the development of AKI is challenging.[5] Once AKI is established, there is often progression to need renal replacement therapy (RRT).[6] Therefore, in patients with decompensated cirrhosis, there is an urgent need to find biomarkers that can (1) identify susceptibility to AKI and/or (2) predict progression to RRT, so that future studies can evaluate prevention or earlier intervention strategies. Studies have shown major changes in metabolomic profiles of serum and urine in patients who have established renal insufficiency without cirrhosis.[7,8] In patients with cirrhosis, single-center outpatient studies have also demonstrated changes in metabolomics in those who died or required liver transplant,[9-11] and in multicenter studies in which renal impairment is already established.[12] However, the role of metabolomics in the prediction of AKI development and its progression to need RRT is unclear from a multicenter perspective.

Our aims were therefore to determine, in hospitalized patients with cirrhosis, whether specific urinary and serum metabolites on admission can predict the development of AKI, and, in those who develop AKI, to prognosticate progression to need dialysis.

Patients and Methods

Patients were enrolled prospectively in 11 centers of the North American Consortium for the Study of End-Stage Liver Disease (NACSELD). They gave samples after informed consent. NACSELD consists of North American tertiary care hepatology centers that have collected prospective data from patients with cirrhosis hospitalized nonelectively, without

HIV infection or prior organ transplants. The study was approved by institutional review boards at all sites. Data were entered in a REDCAP database. For this study, we only included the subset of hospitalized patients who were (1) without AKI or dialysis on admission and (2) who consented to providing serum or urine samples within 12 hours of admission. Patients with pre-existing AKI, those on dialysis on admission, and those who were unable or unwilling to provide samples were excluded from this substudy. All sites were instructed on uniform sample collection practices before study initiation, and samples were stored in −80°C freezers until analysis.

Data pertaining to demographics, cirrhosis details, medications, reasons for admission, and hospital course were recorded. AKI was defined as an acute increase in sCr of ≥0.3 mg/dL within 48 hours or by ≥50% from a stable baseline sCr within 3 months and presumed to have developed within the past 7 days when no prior readings are available.[13] Peak AKI stage was recorded.

Analyses were performed at Metabolon Inc. (Morrisville, NC) using validated ultrahigh-performance liquid chromatography–tandem mass spectroscopy (LC/MS-MS). Analysis of covariance (ANCOVA) analyses were performed adjusting for age, sex, alcohol-associated etiology, admission values of Model for End-Stage Liver Disease (MELD), 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-test were used to determine metabolites that were different between groups. Then an ANCOVA was performed. An estimate of the FDR was calculated to consider the multiple comparisons that normally occur in metabolomic-based studies.[11] Instrument variability was determined by calculating the median relative standard deviation (RSD) for the internal standards that were added to each sample before 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 outcomes of interest (AKI development and need for dialysis) on 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 associated with the outcomes were then explored deeper for each outcome. Random forest analysis (RFA) was then performed, which is a supervised classification technique based on an ensemble of decision trees.[14] For a given decision tree, a random subset of the data with identifying true class information is selected to build the tree without replacement and sample the same number from each group. The in-bag samples are different for each tree. Then after the forest is constructed, the predictions are made for the out-of-bag (OOB) samples for each tree. For each tree, only a subset of variables is considered as determined by the mtry parameter (which is the number of random variables used in each tree "bootstrap sample" or "training set").

The final classification of each sample is determined by computing the class prediction frequency ("votes") for the OOB samples over the whole forest. This method is unbiased, as the prediction for each sample is based on trees built from a subset of samples that do not include that sample. 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 have little change in the accuracy of the class prediction (permuting random noise will give random noise). In 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. AUCs for all metabolites were calculated for the ANCOVA-adjusted models for each category, including those with/without admission infection. Then, we compared urinary and serum metabolomics of the patients with AKI who required dialysis versus those who did not progress to require dialysis using ANCOVA, and RFAs were also performed. Finally, logistic regression models for AKI (yes/no) and dialysis (yes/no) were developed for the clinical variables only (age, admission values of WBC, Na, albumin, and MELD-Na, and infection) and then clinical models plus metabolites significant on RFA. From these models, receiver operator characteristic (ROC) curves were created, and the AUCs with 95% CIs were calculated. Finally, the AUC values for the clinical variables only and combined models were compared using the nonparametric method of DeLong[15] for two or more correlated ROC curves.

Results

OVERALL PATIENT FLOW

We considered a total of 2,403 patients, of whom 105 had AKI on admission and 56 were already on dialysis on admission or at home. Of the remaining 2,242 patients, 527 were transferred in from another hospital, 623 were approached more than 12 hours following admission (as allowed in the NACSELD protocol), and 490 refused to provide serum/urine or were unable to provide urine during this time period. Ultimately, 602 patients who fit the criteria gave serum and 435 patients who fit the criteria gave urine.

CLINICAL COURSE

Of the 602 patients without AKI on admission who provided serum samples, 218 developed AKI 4±2 days following admission, and 80 required dialysis 6±3 days following admission (Table 1 and Supporting Fig. S1). Of these 218 patients, 179 or 82% developed ≥ stage 2 AKI. Patients who developed AKI had similar age, sex, admission spontaneous bacterial peritonitis (SBP) prophylaxis, mean arterial pressure (MAP), and serum albumin, but had worse cirrhosis severity by MELD scores, higher prevalence of ascites and HE, higher rate of admission in the past 6 months, infections as the reason for admission, and higher admission WBC, compared with those who did not develop AKI. Admission sCr, MAP, statin and nonselective beta-blocker (NSBB) use were similar. Patients with AKI had a higher rate of acute-on-chronic liver failure (ACLF) development as defined by NACSELD,[16] a longer hospital length of stay (LOS), a higher rate of intensive care unit (ICU)

admission, and a higher rate of death versus those who did not. Patients who required dialysis again had similar demographics, admission SBP prophylaxis, NSBB and statin use, and admission WBC compared with patients who did not require dialysis but had a higher rate of admission infections, higher admission MELD score and sCr and lower MAP, and worse inpatient outcomes (development of ACLF, ICU admissions, LOS, and death).

Of the 435 patients without AKI on admission who provided urine samples, 164 developed AKI 4±2 days following admission, and 61 patients required dialysis 6±2 days following admission (Table 1 and Fig. 1). As found in serum, most patients with AKI developed stage 2 or higher disease (n = 139, 79%). Patients who provided urine samples and who developed AKI had similar age, sex, admission SBP prophylaxis, MAP, and serum albumin, but had worse cirrhosis severity (admission MELD score and prevalence of ascites and HE), and higher rate of admission infections and admission WBC, which resulted in a higher percentage of patients who developed ACLF, had a longer hospital LOS, and a higher mortality when compared with those who did not develop AKI. When comparing patients with AKI who progressed to need for dialysis (n = 61) versus those who did not (n = 103), once again they had similar demographics, admission serum creatinine, admission SBP prophylaxis, NSBB and statin use, and serum albumin, but had a greater infection rate and higher admission WBC, worse cirrhosis severity, and worse outcomes (ACLF development, ICU admission, LOS, and death). None of the patients were on vasopressors on admission. The peak sCr was higher in those with renal outcomes, regardless of the cohort studied.

PREDICTION OF AKI DEVELOPMENT

In the entire group (n = 602 with serum samples and n = 435 with urine samples), ANCOVA analysis adjusted for age, gender, alcohol-associated etiology and admission WBC, Na, albumin, and MELD score showed multiple metabolites in serum and urine that differentiated between those who developed AKI compared with those who did not. These metabolites spanned all classes but aromatic and branched chain amino acids (BCAAs), urea cycle and dipeptides, and bile acids, along with products of purine and pyrimidine metabolism, were major contributors toward this difference. Prediction of AKI development had an AUC of 0.91 based on serum metabolites and 0.88 based on urine metabolites adjusted for the clinical variables using ANCOVA. The top metabolites that were relevant in the MDA analysis are shown in Fig. 2. The OOB values for AKI prediction was 0.3 for both serum and urine, implying that the error rate of these metabolites in predicting AKI was 30%. Of these, the direction of metabolites in those who developed outcomes versus those who did not are given in Table 2. Several known uremic toxins or metabolites that are known to be increased in patients without cirrhosis with renal insufficiency were higher in our study cohort who developed AKI, and in all of them increased before sCr increased, and the clinical diagnosis of AKI was established. In addition, potentially beneficial metabolites such as homoarginine and BCAA were reduced. We also found changes in lipid moieties (bile acids and phospholipids) that were perturbed in patients with AKI. Representative metabolite least squares (LS) mean comparisons are shown in Fig. 2 and Supporting Tables S1 and S2. Infected/uninfected patients were analyzed separately using all metabolites for AKI prediction. For serum, the AUC in the uninfected group was 0.81 and in the infected group was 0.82, while in urine the AUC for the uninfected patients was 0.76 and in the

infected patients it was 0.79. Due to the relatively low number of people who required dialysis either in the entire group or within AKI subgroups, this calculation was not possible for that outcome by splitting the data set.

PREDICTION OF DIALYSIS INITIATION IN THE ENTIRE GROUP

Among all patients included in the study (n = 602 serum and n = 435 urine), there were metabolites that differentiated patients who required dialysis versus those who did not. Prediction of dialysis requirement had an AUC of 0.93 for serum and 0.91 for urine metabolites, which was adjusted for clinical variables with ANCOVA. The OOB values for RFA for dialysis requirement were 0.2 for both serum and urine, meaning that the error rate for dialysis prediction was 20%. As with the results for AKI development, these metabolites were from amino acid (cysteine, tryptophan, tyrosine), purine/pyrimidine metabolism, and uremic toxins whose relative changes in LS means and direction of change are shown in Fig. 3 and Supporting Tables S3 and S4). Similar changes in serum and urine to those seen in AKI prediction were also identified on RFA and ANCOVA analyses.

PREDICTION OF DIALYSIS INITIATION IN AKI SUBGROUP

In the patients who developed AKI, the analysis of differences in metabolites between those who did or did not require dialysis was performed. The OOB values for both serum and urine AKI with random forest development was 0.3. The AUC for urine metabolites to predict who required dialysis was 0.79, whereas it was 0.81 for serum metabolites. The major changes in direction of relevant metabolites between these groups are given in Table 3, and representative metabolite LS mean changes are displayed in Fig. 4 and Supporting Tables S5 and S6.

COMPARISON WITH CLINICAL MODEL

As indicated in Table 4, the addition of metabolites different on RFA significantly increased the AUC of outcomes prediction of AKI and dialysis in the entire group and of dialysis in the group with AKI in both serum and urine.

Discussion

Biomarkers for the prediction of or early detection of AKI and progression to dialysis are critically important for earlier implementation of therapy and prognostication in patients with decompensated cirrhosis.[1,17] Using data and samples from a multicenter cohort of hospitalized patients with cirrhosis, we demonstrate that specific panels of metabolites in urine and serum obtained on hospital admission can predict the development of AKI in those without it on admission, as well as progression to dialysis in those who developed AKI.

Sarcopenia and variations in muscle mass between sexes makes the status quo for diagnosing AKI challenging in cirrhosis.[18] Studies have shown that even relatively minor changes in sCr can portend higher short-term mortality even after reversal.[19] Therefore, prevention of AKI development and earlier institution of treatment are urgently needed. Unfortunately, biomarkers to predict AKI development that are available clinically have yet to be developed and validated. These are of critical importance for intervention strategies

before AKI develops and prognosis worsens. Although focused studies on urinary and serum biomarkers such as neutrophil gelatinase–associated lipocaliin, kidney injury molecule 1, IL-18, and liver type fatty acid binding protein have been reported, a more expansive view that considers the multiple system alterations inherent in patients with advanced cirrhosis is needed.[2,4] Therefore, untargeted metabolomics of serum and urine are important tools in agnostically determining patterns of metabolic dysfunction, which can guide diagnosis, prognosis, and improve insight into the pathophysiology of disease.

Biomarkers of kidney disease can be those related to kidney damage, inflammation, retention of metabolites that should have been excreted, secretion changes in metabolites due to injury, or a combination of these.[5] The liver and kidney individually impact systemic metabolism; therefore, injuries to both organs makes it more challenging and complicated to interpret biomarkers.

Our analysis demonstrates that key metabolites belonging to aromatic and BCAA and cysteine/methionine metabolism, known uremic toxins, and lipids can predict and detect the development of AKI and the need for dialysis in those with and without AKI in inpatients with cirrhosis. These data provide a framework for us to explore proactive strategies to prevent AKI and need for dialysis in hospitalized patients by identifying this at-risk group.

Metabolite changes showed robust AUC values in serum and urine for prediction even after adjusting for clinically relevant biomarkers. Specific metabolites include uremic toxins and substances that parallel glomerular filtration rate (GFR) reduction such as 2,3-dihydroxy-5-methylthio-4-penten oic acid (DMTPA), N,N,N-trimethyl-L-alanyl-L-proline betaine (TMAP), N2-N2 dimethylguanosine, C-glucosyltryptophan and pseudouridine, which are associated with kidney function regardless of cirrhosis, were higher in those that developed AKI and required dialysis.[7,20-24] Major metabolites belonging to aromatic amino acid metabolism (tryptophan: kynunerine, 8-methoxykynunerate; tyrosine, 3-(4-hydroxyPhenyl) lactate, homovanillate sulfate, vanillactic, and hydroxyphenyllactic acid) were also higher in patients who developed AKI. These metabolites largely reflect the excretion ability of the kidney and parallel the GFR reduction regardless of etiology of kidney disease. Our analysis extends the importance of these metabolites in hospitalized patients with cirrhosis, as they reflect not only the current but the future risk for development of negative renal outcomes despite adjustment for age, gender, and cirrhosis severity. This underlines the need to better prognosticate these outcomes than our current biomarkers.

The catabolism of S-adenosyl methionine toward polyamine synthesis generates methylthioadenosine, which is a precursor of DMTPA. S-adenosyl methionine is also the precursor for S-adenosylhomocysteine (SAH) and cystathionine. Cystathionine in serum was associated with only AKI development but not the need for dialysis, whereas SAH was one of the strongest predictors for AKI development and dialysis initiation in the urine and the serum. SAH was higher in those who required dialysis, whether the entire group of patients or just those with AKI was used as the denominator, indicating the wide applicability of the data. The specific metabolites are in line with Mindikoglu et al. and further extend them into a multicenter realm using both prospectively collected serum and urine samples.[9] The metabolomic signature detected reflects the beginning of the bio-energetic and amino acid

disruption that has been published in prior studies in which renal failure has already been established.[12,25]

In addition to excretory markers, we also found profound changes in potential secretory markers that are influenced by multiple systems in patients who developed AKI and needed dialysis. Our findings focused on tryptophan metabolism leading to kynunerate, anthranilate, quinolinate, and picolinate formation. These metabolites can be generated from dietary tryptophan and converted to kynunerate in the liver or (12)peripheral organs, such as the kidney.[7] The role of the kidneys in tryptophan metabolism is complex and can result in excretion of derivatives and production of kynunerine pathway metabolites. In rodent models of renal failure, only renal and not hepatic generation of kynunerine is increased.[26] The accumulation and excess of these metabolites result in mitochondrial dysfunction and lead to neurological, vascular, and lipid metabolic impairments.[27] In patients with cirrhosis, Claria et al. reaffirmed this in patients with renal failure in the setting of ACLF, in which elevated tryptophan metabolites were associated with greater mortality.[28]

Our study extends these data into a predictive analysis of secreted and accumulated metabolites in cirrhosis. In addition to tryptophan, several other aromatic amino acid metabolites were higher in those who developed AKI or required dialysis. Consistent among these were vanillactate, homovanillate sulfate and hydroxyphenyllactate moieties, which are tyrosine and levodopa degradation products.[8] Vanillactate and homovanillate sulfate are stress markers that we found to be higher in those with negative consequences, regardless of whether the entire group or the AKI subgroup was considered.[8] In addition to the compounds that were higher and reflect accumulation, kidney damage or excess secretion, certain other metabolites that are usually associated with benefit such as BCAA derivatives (leucine leading to methylmalonic and valine leading to beta-hydroxy isovalerate), homoarginine[29] and lipid moieties (phospholipids and androgens) were lower in those who developed AKI and needed dialysis.[11,30,31] These findings show that the altered metabolites detected are not just increased by functional accumulation and excessive renal secretion, but also decreased beneficial metabolites that protect against sarcopenia and cell membrane instability.

We also focused on the AKI-only group to assess whether we could predict who improved versus progressed to require dialysis. Although the AUCs were greater than 0.79 for both serum and urine samples, the smaller sample size was a limitation. Despite this, we identified several metabolites that could predict which AKI patients would eventually need versus not need dialysis. Several of these compounds (vanillactate, DMTPA, C-glycosyl tryptophan, kynunerate, 8-methoxykynunerate, and SAH) were similar to what was seen for dialysis prediction in the entire group. This validates the importance of these specific metabolites in the progression to dialysis. However, there were some other metabolites that were unique to this subgroup, including other catecholamine degradation products (vanillylmandelic acid) and N-acetylated/carbamolyated amino acids, which have been independently associated with renal function in patients with and without cirrhosis.[9,32] In addition, arginine derivatives (asymmetric dimethylarginine [ADMA]/symmetric dimethylarginine [SDMA]) and tryptophan metabolite from the serotonin pathway (5-hydroxyindoleacetate 5HIAAA) were also higher. ADMA/SDMA are associated with

vascular reactivity, portal hypertension, and brain dysfunction in cirrhosis and are produced by the kidneys in health and increase with disease.[33] 5HIAAA in addition to kynunerine represents further tryptophan breakdown in both these pathways and again underlines the importance of tryptophan metabolism in patients with cirrhosis and renal insufficiency.

The study's strengths are the multicenter nature of the data, collection of urine and serum samples within 12 hours of admission using uniform techniques, use of robust LC/MS-MS metabolomic technology, and narrowing down the significant metabolites that were additive to clinical variables. In addition, once validated, the predictive nature of these metabolites before the sCr increases could potentially guide clinicians to initiate preventive therapy, remove nephrotoxic agents earlier, and monitor these patients closer. These metabolomic data reflect the several pathways that are affected in this complex group of inpatients with cirrhosis and impending AKI and possible need for dialysis. The untargeted nature of our approach identified several metabolic derangements, which provides a greater overall view of the alterations and reduced the multiple compounds discovered to the most predictive few significant metabolites. These particular markers are typically less dependent on age and sex than the usual biomarkers such as sCr.[34] They also are distinct from what we found in the same population as predictors of ACLF and death in the hospital.[35] This indicates that these metabolites are specific to AKI and dialysis development, rather than markers for a generally poor prognosis in this inpatient cirrhosis population.

The study's weaknesses include (1) the use of samples from all AKI types and severities rather than separating the group into subtypes (i.e., AKI–hepatorenal syndrome [HRS] vs. AKI–non-HRS) or severities, although most were at least stage 2 at peak AKI stage. Given the diagnostic dilemma frequently present when trying to subdivide AKI types in patients with cirrhosis combined with our limited numbers, further subdivision was not possible. (2) We used requirement for dialysis, which can vary between centers and by the patient's transplant candidacy. However, we found consistent changes in metabolites. (3) Of the 602 patients who provided serum samples and 435 who provided urine samples, only 286 gave both samples, which is a relatively low sample size for metabolomics-related outcome modeling. Therefore, the analysis was done separately, but we found similar metabolites that were important in AKI and dialysis requirement prediction in serum and urine. (4) Serum-based metabolomics was better at predicting AKI and need for dialysis than urine, but it could also be due to the larger sample size of patients who provided serum. This also demonstrates that only one biofluid may be enough to predict these outcomes.

This experience is the a step in developing a serum or urine metabolomic profile to predict the development and progression of AKI. Our data need to first be replicated in other cohorts before specific metabolites can be translated into laboratory panels for point-of-care diagnostics. Future advances need to focus on finding biomarkers to identify susceptibility, mechanism of injury, and response to treatment. The use of metabolomics, if validated, could result in earlier prediction and diagnosis, which can enable earlier intervention to improve renal function and subsequent prognosis.

We conclude that serum and urinary metabolites, especially those involved in the catabolism of S-adenosyl methionine or tryptophan metabolism, can predict the development of AKI

and requirement for dialysis in a multicenter cohort of inpatients with cirrhosis. Further validation and potential translation of these metabolite changes may be important to initiate point-of-care diagnostics to guide management of patients to prevent AKI and progression toward requirement of renal replacement.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

5-HIAAA	5-hydroxyindoleacetate
ACLF	acute on chronic liver failure
ADMA	asymmetric dimethylarginine
AKI	acute kidney injury
ANCOVA	analysis of covariances
BCAA	branched chain amino acid
DMTPA	2,3-dihydroxy-5-methylthio-4-pentenoic acid
FDR	false discovery rate
ICU	intensive care unit
LOS	length of stay
LS means	least square means
MAP	mean arterial pressure
MDA	mean decrease accuracy
MELD	Model for End-Stage Liver Disease
NACSELD	North American Consortium for the Study of End-Stage Liver Disease
NSBB	nonselective beta blocker
OOB	out-of-bag
RFA	random forest analysis

RRT	renal replacement therapy
RSD	relative SD
SAH	S-adenosylhomocysteine
SBP	spontaneous bacterial peritonitis
sCr	serum creatinine
SDMA	symmetric dimethylarginine
TMAP	N,N,N-trimethyl-L-alanyl-L-proline betaine
WBC	white blood count

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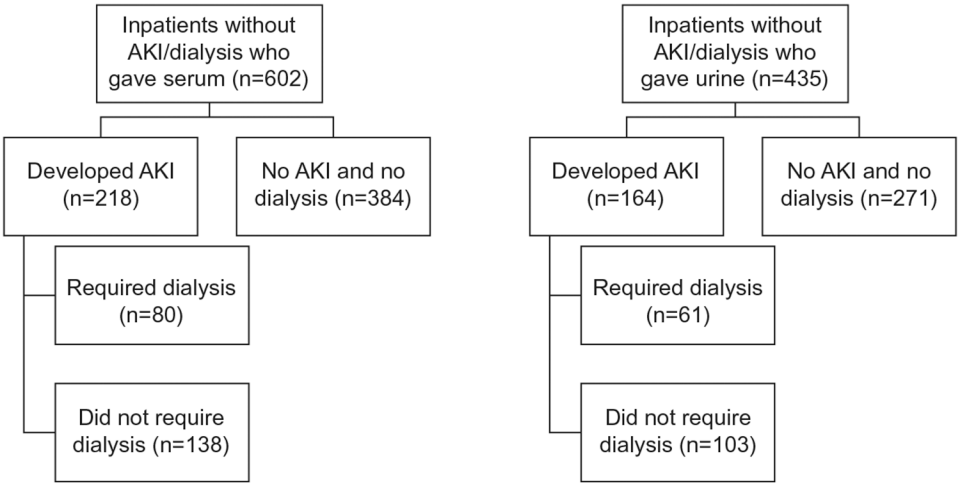

FIG. 1.
Patient flow after entry into the study.

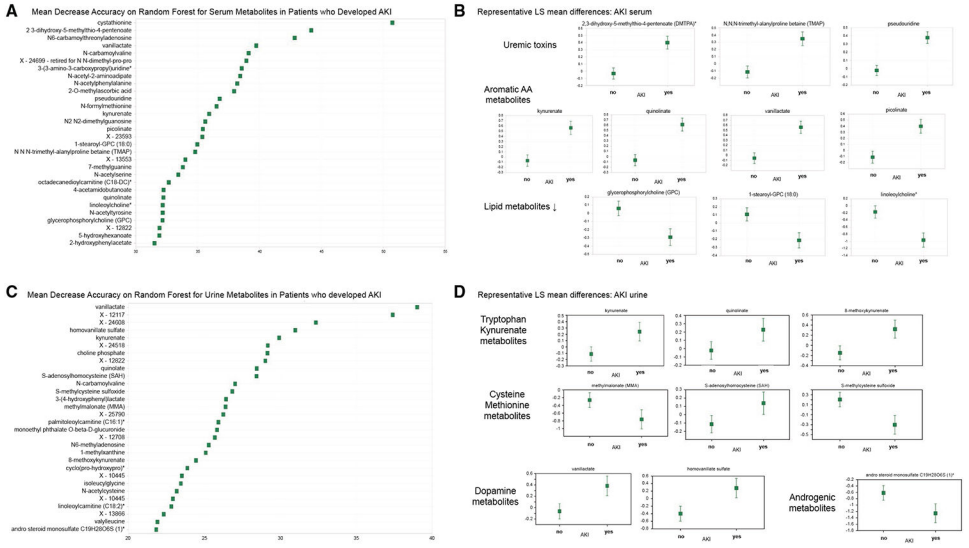

FIG. 2.

Random forest mean decrease accuracy for AKI development using serum and urine metabolites for the entire group. (A) Mean decrease accuracy on random forest for serum metabolites in patients who developed AKI. (B) Representative LS mean differences: AKI serum yes or no. (C) Mean decrease accuracy on random forest for urine metabolites in patients who developed AKI. (D) Representative LS mean differences: AKI urine yes or no.

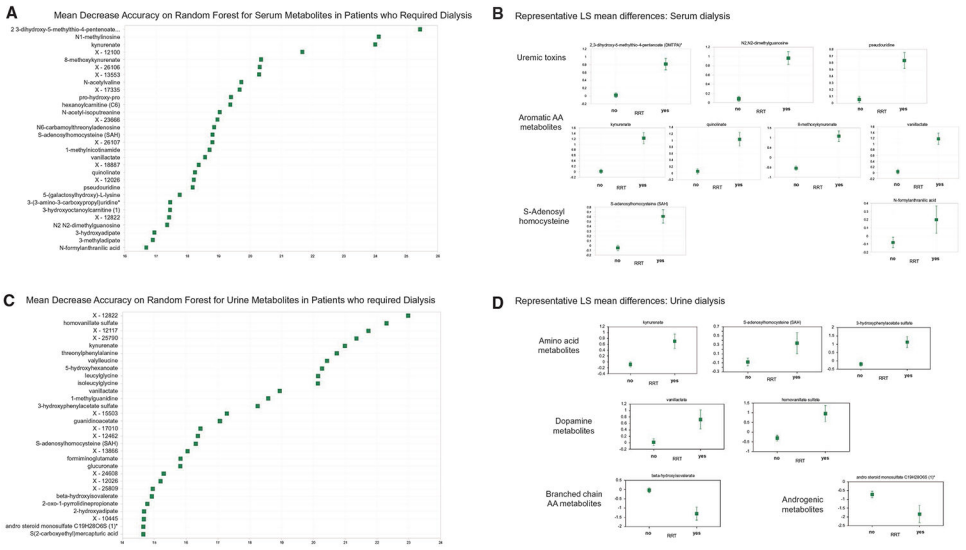

FIG. 3.

Random forest mean decrease accuracy for dialysis (RRT) requirement using serum and urine metabolites for the entire group. (A) Mean decrease accuracy on random forest for serum metabolites in patients who required RRT. (B) Representative LS mean differences: serum yes or no. (C) Mean decrease accuracy on random forest for urine metabolites in patients who required RRT. (D) Representative LS mean differences: urine yes or no.

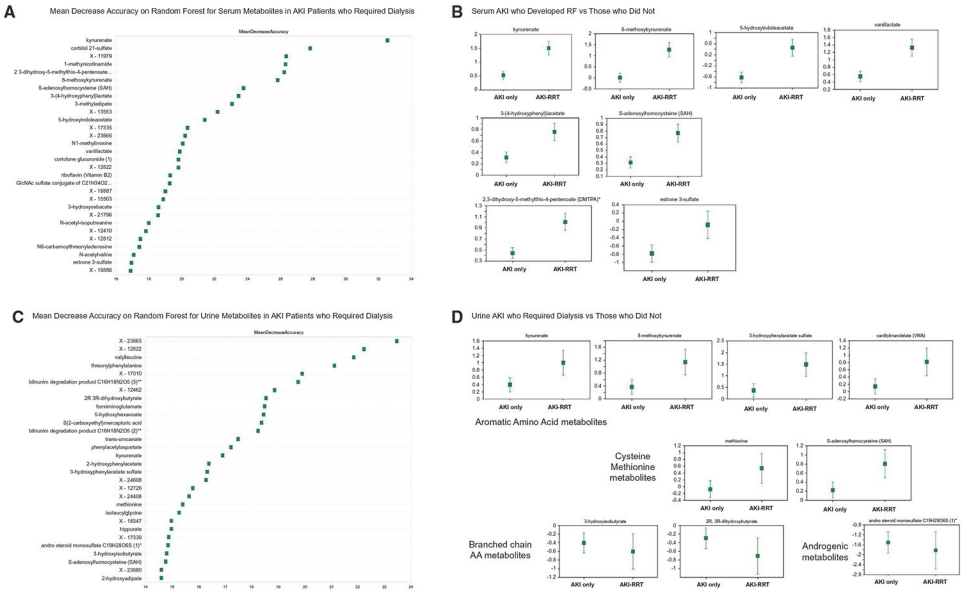

FIG. 4.

Random forest mean decrease accuracy for dialysis requirement using serum and urine metabolites for the subgroup with AKI. (A) Mean decrease accuracy on random forest for serum metabolites in patients who required dialysis (AKI-RRT) within the AKI group. (B) Representative LS mean differences: serum, yes or no. (C) Mean decrease accuracy on random forest for urine metabolites in patients who required dialysis (AKI-RRT) within the AKI group. (D) Representative LS mean differences: urine yes or no.

TABLE 1.

Clinical Comparisons Between Patients Who Developed AKI or Who Required Dialysis

	Serum Metabolites: AKI (n = 602)			Urine Metabolites: AKI (n = 435)			Serum Metabolites: Dialysis Placement (n = 602)			Urine Metabolites: Dialysis Placement (n = 435)		
Variable	**No (n = 384)**	**Yes (n = 218)**	***P* Value**	**No (n = 271)**	**Yes (n = 164)**	***P* Value**	**No (n = 522)**	**Yes (n = 80)**	***P* Value**	**No (n = 374)**	**Yes (n = 61)**	***P* Value**
Age (years)	56.1 ± 9.4	56.2 ± 10.2	0.85	55.9 ± 9.2	55.9 ± 8.5	0.94	56.2 ± 9.5	54.7 ± 10.1	0.22	56.0 ± 9.4	54.8 ± 8.8	0.31
Men (n [%])	246 (64%)	128 (59%)	0.28	192 (71%)	105 (64%)	0.13	315 (60%)	59 (74%)	0.71	255 (68%)	42 (69%)	0.32
Diabetes (n [%])	123 (32%)	74 (34%)	0.63	86 (32%)	49 (30%)	0.69	172 (33%)	25 (31%)	0.76	124 (33%)	21 (34%)	0.85
Etiology: alcohol/HCV/	107/125/	70/75/	0.12	84/110/50/27	55/53/	0.36	150/172	27/28/	0.47	121/138/	18/25	0.47
NASH/other	69/83	43/30			37/19		/102/98	10/15		78/37	/9/9	
MELD score	16.9 ± 6.4	23.5 ± 8.1	<0.001	17.1 ± 6.3	24.0 ± 7.8	<0.001	18.2 ± 7.2	27.6 ± 6.5	<0.001	18.4 ± 7.0	27.9 ± 6.4	<0.001
Serum Na (meq/L)	134.6 ± 6.0	132.3 ± 6.0	<0.001	134.5 ± 6.1	132.1 ± 5.8	<0.001	134.1 ± 5.9	131.5 ± 6.7	0.001	134.0 ± 5.9	131.6 ± 6.8	0.01
Admission creatinine (mg/dl)	1.15 ± 0.72	1.25 ± 0.65	0.08	1.14 ± 0.73	1.31 ± 0.98	0.06	1.14 ± 0.97	1.72 ± 1.49	0.001	1.13 ± 0.75	1.82 ± 1.14	0.001
Prior ascites (n [%])	285 (74%)	185 (85%)	<.001	193 (71%)	133 (81%)	0.02	398 (76%)	72 (90%)	<0.001	277 (74%)	49 (80%)	0.29
Admission MAP (mmHg)	86.4 ± 17.3	83.9 ± 15.2	0.07	87.0 ± 15.0	84.8 ± 14.0	0.12	86.4 ± 14.3	82.7 ± 13.6	0.03	86.5 ± 14.7	83.7 ± 14.2	0.15
Serum albumin (g/dL)	2.9 ± 0.6	2.8 ± 0.7	0.23	2.8 ± 0.6	2.7 ± 0.7	0.14	2.8 ± 0.6	2.9±0.8	<0.001	2.8 ± 0.7	2.9 ± 0.8	0.48
WBC ($\times 10^3$/mL)	7.1 ± 4.1	9.5 ± 5.8	<0.001	7.2 ± 4.2	9.8 ± 5.9	<0.001	7.5 ± 4.7	10.4 ± 5.6	0.29	7.8 ± 4.9	10.1 ± 5.3	0.002
Admission infection (n [%])	107 (28%)	104 (48%)	0.01	105 (39%)	81 (49%)	0.05	182 (35%)	54 (68%)	<0.001	154 (41%)	42 (69%)	<0.001
Admission HE (n [%])	143 (37%)	130 (60%)	<0.001	96 (35%)	76 (46%)	0.02	221 (59%)	52 (65%)	0.002	131 (35%)	41 (67%)	<0.0001
Admission rifaximin (n [%])	134 (35%)	124 (57%)	<0.001	89 (33%)	68 (41%)	<0.001	210 (40%)	48 (60%)	0.009	123 (33%)	34 (56%)	0.001
Admission statins	34 (9%)	20 (9%)	0.91	24 (9%)	15 (9%)	0.91	48 (9%)	6 (7%)	0.66	36 (14%)	3 (8%)	0.33
Admission NSBB	172 (44%)	87 (40%)	0.27	108 (40%)	70 (43%)	0.56	221 (43%)	38 (48%)	0.38	159 (43%)	19 (31%)	0.10
Admission SBP prophylaxis (n [%])	35 (9%)	24 (11%)	0.52	15 (6%)	11 (7%)	0.39	31 (6%)	5 (6%)	0.37	26 (7%)	4 (7%)	0.37
Peak creatinine (mg/dL)	1.29 ± 0.89	2.71 ± 1.77	<0.001	1.31 ± 0.91	2.61 ± 1.73	<0.001	1.50 ± 1.03	5.01 ± 2.01	<0.001	1.51 ± 1.03	5.06 ± 1.99	<0.001
ACLF (n [%])	14 (4%)	66 (30%)	<0.001	14 (5%)	48 (29%)	<0.001	32 (6%)	56 (70%)	<0.001	26 (7%)	43 (70%)	<0.001
Inpatient death n (%)	4 (1%)	35 (16%)	<0.001	2 (13%)	23 (14%)	<0.001	16 (3%)	25 (31%)	<0.001	12 (3%)	16 (26%)	<0.001
ICU transfer (n [%])	38 (10%)	84 (39%)	<0.001	36 (13)	55 (36%)	<0.001	91 (17%)	53 (67%)	<0.001	67 (18%)	37 (61%)	<0.001
LOS (days)	9.2 ± 46.1	18.0 ± 19.7	0.005	7.2 ± 13.1	14.0 ± 11.9	<0.001	8.9 ± 9.9	29.7 ± 33.0	<0.001	7.8 ± 8.1	23.9 ± 26.7	<0.001

Note: Data are presented as mean ± SD or in raw numbers (%). Comparisons were performed using unpaired Student t tests or Mann-Whitney U tests as appropriate. All laboratory values are on admission to hospital.

TABLE 2.

Metabolites in the Entire Group With Highest Mean Decrease Accuracy on RFA Between Those Who Developed Outcomes and Those Who Did Not

Serum AKI vs. Not	↑/↓	Urine AKI vs. Not	↑/↓	Serum Dialysis vs. Not	↑/↓	Urine Dialysis vs. Not	↑/↓
Cystathionine	↑	vanillactate	↑	DMTPA	↑	homovanillate sulfate	↑
DMTPA	↑	homovanillate sulfate	↑	N1-methylinosine	↑	kynurenate	↑
N6-carbamoylthreonyl adenosine	↑	kynurenate	↑	kynurenate	↑	threonlyphenyl alanine	↑
Vanillactate	↑	choline phosphate	↑	8-methoxykynurenate	↑	valylleucine	↑
3-(3-amino-3-carbopropoxyl) Uridine	↑	quinolinate	↑	N-acetyl valine	↑	5-hydroxyhexanoate	↓
N-acetyl-2-aminoadipate	↑	S-adenosyl homocysteine	↑	Pro-hydroxy-pro	↑	Leucylglycine	↑
N-acetylphenylalanine	↑	N-carbamoyl valine	↑	hexanoyl-carnitine	↑	isoleucylglycine	↑
2-O-methylascorbic acid	↑	S-methyl cysteine sulfoxide	↓	N-acetyl isoputreanine	↑	vanillactate	↑
Pseudouridine	↑	3-(4-hydroxy phenyl) lactate	↑	N6-carbamoyl-threonyl adenosine	↑	1-methyl guanidine	↑
N-formylmethionine	↑	methyl malonic acid	↓	1-methylnicotinamide	↑	3-hydroxy phenylacetate sulfate	↑
Kynurenate	↑	palmitoleoyl carnitine (16:1)	↑	vanillactate	↑	guanidinoacetate	↓
N2 N2-dimethylguanosine	↑	monoethyl phthalate O-beta-D-glucuronide	↑	quinolinate	↑	S-adenosyl homocysteine	↑
Picolinate	↑	N6-methyl adenosine	↓	pseudouridine	↑	formiminoglutamate	↑
1-stearoyl-GPC	↓	1-methyl xanthine	↓	5-(galactosyl hydroxy)-L-lysine	↑	glucuronate	↑
TMAP	↑	8-methoxykynurenate	↑	3-(3-amino-carboxypropyl) uridine	↑	beta-hydroxy isovalerate	↓
7-methylguanine	↑	cyclo(prohydroxy-pro)	↑	3-hydroxyoctanoyl carnitine	↑	2-oxo-1-pyrrolidine propionate	↑
Octadecanediolycarnitine	↑	isoleucylglycine	↑	N2 N2-dimethyl guanosine	↑	2-hydroxyadipate	↑
4-acetamidobutanoate	↑	N-acetylcysteine	↑	3-hydroxy adipate	↑	androsteroid mono sulfate	↓
Quinolinate	↑	linoleoylcarnitine (16:2)	↑	3-methyl adipate	↑	S(2-carboxyethyl) mercapturic acid	↑
lineloylcholine	↓	valylleucine	↑	N-formyl anthranilic acid	↑		
N-acetyltyrosine	↑	Androsteroid monosulfate	↓				
Glycerlyphosphocholine	↓						
5-hydroxyhexanoate	↑						
2-hydroxyphenylacetate	↑						

Note: Metabolites are listed in order of mean decrease accuracy on RFA. ↑, higher in those who developed outcomes compared with those who did not, and vice versa for ↓.

TABLE 3.

Metabolites Within the AKI Group With Highest Mean Decrease Accuracy on RFA Between Those Who Required Dialysis Versus Those Who Did Not

Serum AKI Leading to Dialysis Requirement vs. Not	↑/↓ in Dialysis	Urine AKI Leading to Dialysis Requirement vs. Not	↑/↓ in Dialysis
Kynurenate	↑	beta-citrylglutamate	↑
8-methoxykynurenate	↑	C-glycosyltryptophan	↑
DMTPA	↑	3-hydroxyphenylacetate sulfate	↑
5-hydroxyindoleacetate	↑	isoputreanine	↑
vanillactate	↑	N-acetyl-isoputreanine	↑
S-adenosylhomocysteine	↑	8-methoxykynurenate	↑
3-methylglutaconate	↑	N1,N12-diacetylspermine	↑
Methylsuccinate	↑	aspartate	↑
3-(4-hydroxyPhenyl)lactate	↑	S-adenosylhomocysteine	↑
N-acetyl-isoputreanine	↑	N-acetyputrescine	↑
Dimethylarginine (ADMA/SDMA)	↑	xanthurenate	↑
5-(galactosylhydroxy)-L-lysine	↑	cystine	↑
Vanillylmandelate	↑	N-acetyl-aspartyl-glutamate	↑
N-acetyltaurine	↑	beta-hydroxyisovalerate	↓
N-acetylvaline	↑	vanillylmandelate	↑
1-methylguanidine	↑	fructosyllysine	↑
Beta-citrylglutamate	↑	kynurenate	↑
3-amino-2-piperidone	↑	gamma-carboxyglutamate	↑
Succinoyltaurine	↑	isovalerylglutamine	↑
C-glycosyltryptophan	↑	lanthionine	↑
4-acetamidobutanoate	↑	N-carbamoylvaline	↑
Methylsuccinoylcarnitine	↑	formiminoglutamate	↑
N-acetylalanine	↑	DMTPA	↑
N-formylmethionine	↑	beta-citrylglutamate	↑

Note: Metabolites are listed in order of mean decrease accuracy on RFA. ↑, higher in those who developed outcomes compared with those who did not, and vice versa for ↓.

TABLE 4.

Comparisons of AUCs Between Models Containing Clinical Variables Only and Models With Clinical Variables and Metabolites

	All Subjects						
Biofluid	**Serum (n = 602)**		**Urine (n = 435)**		**Biofluid**	**Serum (n = 218)**	**Urine (n = 164)**
Outcome	**AKI**	**Dialysis**	**AKI**	**Dialysis**	**Outcome**	**Dialysis**	**Dialysis**
AUC with clinical variables only	0.76 (0.72, 0.80)	0.84 (0.80, 0.88)	0.79 (0.73, 0.83)	0.82 (0.75, 0.87)	AUC with clinical variables only	0.74 (0.67, 0.81)	0.71 (0.61, 0.79)
AUC with clinical variables plus metabolites	0.86 (0.83, 0.89)	0.93 (0.89, 0.95)	0.85 (0.80, 0.89)	0.94 (0.91, 0.96)	AUC with clinical variables plus metabolites	0.87 (0.81, 0.92)	0.94 (0.89, 0.97)
Difference	−0.10 (−0.14, −0.17)	−0.09 (−0.12, −0.05)	−0.06 (−0.10, −0.03)	−0.12 (−0.17, −0.07)	Difference	−0.13 (−0.19, −0.07)	−0.23 (−0.33, −0.14)
χ^2	31.4099	19.6499	14.4068	24.0935	χ^2	16.0748	23.3219
P value	<0.0001	<0.0001	<0.0001	<0.0001	*P* value	<0.0001	<0.0001
Individual metabolites that are significantly additive	• cystathionine • N6-carbamoyl threonyl adenosine • 3-(3-amino-3-carbo propoxyl) uridine • N-formylmethionine • kynurenate	• 8-methoxy kynurenate • N-acetyl valine • 1-methyl nicotinamide • quinolinate	• choline phosphate • S-methyl cysteine sulfoxide	• formimino glutamate • beta-hydroxy isovalerate	• Individual metabolites that are significantly additive	• DMPTA	• C-glycosyl tryptophan • N-acetyputrescine • Beta-hydroxy isovalerate • Formimino • glutamate

Clinical variables used: age, admission values of WBC, serum sodium, serum albumin, and MELD-Na and infection.