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ARTICLE

Lipidomic changes in a novel sepsis outcome-based analysis reveals potent pro-inflammatory and pro-resolving signaling lipids

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Abstract

The purpose of this study was to investigate changes in the lipidome of patients with sepsis to identify signaling lipids associated with poor outcomes that could be linked to future therapies. Adult patients with sepsis were enrolled within 24h of sepsis recognition. Patients meeting Sepsis-3 criteria were enrolled from the emergency department or intensive care unit and blood samples were obtained. Clinical data were collected and outcomes of rapid recovery, chronic critical illness (CCI), or early death were adjudicated by clinicians. Lipidomic analysis was performed on two platforms, the Sciex™ 5500 device to perform a lipidomic screen of 1450 lipid species and a targeted signaling lipid panel using liquid-chromatography tandem mass spectrometry. For the lipidomic screen, there were 274 patients with sepsis: 192 with rapid recovery, 47 with CCI, and 35 with early deaths. CCI and early death patients were grouped together for analysis. Fatty acid (FA) 12:0 was decreased in CCI/early death, whereas FA 17:0 and 20:1 were elevated in CCI/early death, compared to rapid recovery patients. For the signaling lipid panel analysis, there were 262 patients with sepsis: 189 with rapid recovery, 45 with CCI, and 28 with early death. Pro-inflammatory signaling lipids from ω-6 poly-unsaturated fatty acids (PUFAs), including 15-hydroxyeicosatetraenoic (HETE), 12-HETE, and 11-HETE (oxidation products of arachidonic acid [AA]) were elevated in CCI/early death patients compared to rapid recovery. The pro-resolving lipid mediator from ω-3 PUFAs, 14(S)-hydroxy docosahexaenoic acid (14S-HDHA), was also elevated in CCI/early death compared to rapid recovery. Signaling lipids of the AA pathway were elevated in poor-outcome patients with sepsis and may serve as targets for future therapies.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

There have been many studies that have investigated the role of phospholipids and their potent downstream metabolites—particularly those derived from arachidonic acid (AA)—in patients with sepsis. Although prior studies of the changes in the lipidome of patients with sepsis have been explored, there were many limitations, namely: (1) small patient sample sizes, (2) lipidomic changes by sepsis severity were not reported, and (3) targeted analyses of pro-inflammatory and pro-resolving signaling lipid species have not been studied.

WHAT QUESTION DID THIS STUDY ADDRESS?

Our study addresses these limitations by investigating the changes in the lipidome of 283 patients with sepsis of varying severity—rapid recovery versus chronic critical illness (CCI) or early death—and compares them with healthy controls. We hypothesized that pro-inflammatory and pro-resolving signaling molecules derived from AA would be elevated in patients with CCI or early death—who were grouped together as the poor outcome group—versus the rapid recovery patients.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

We identified three free fatty acids and four significantly altered pro-inflammatory and pro-resolving signaling lipids (12-HETE, 15-HETE, 11-HETE, and 14S-HDHA) of the AA pathway that were significantly altered between rapid recovery and CCI/early death patients.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Future studies targeting the 12/15-LOX pathway of AA metabolism may be important to improving sepsis outcomes in a subset of patients.

INTRODUCTION

Sepsis is a life-threatening dysregulated response to infection, that leads to organ dysfunction and potentially death.[1] Previously reported sepsis outcomes by our group and others include rapid recovery (clinical improvement and hospital discharge within 14 days), early death (in-hospital death within 14 days), and chronic critical illness (CCI; intensive care unit [ICU] stay of at least 14 days with manageable organ dysfunction).[2] Each clinical trajectory is associated with relevant long-term consequences, particularly for patients with CCI who experience 1-year mortality of nearly 50% in the majority of cases.[2]

Lipid changes during sepsis have been widely reported by our group and others, and have been linked to prognosis and mortality.[3–8] In particular, phospholipids may influence and are associated with sepsis severity and outcomes.[3] Phospholipids are composed of a hydrophilic, polar head group, with esterified fatty acid (FA) tails of various lengths and are either saturated or unsaturated. These phospholipids are then acted on enzymatically through secretory phospholipases—which are increased during sepsis—that then hydrolyze the release of FA tails.[3] Of particular importance are polyunsaturated fatty acids (PUFAs), which play unique roles to either help resolve the inflammatory cascade during sepsis (ω-3 PUFAs) or enhance the inflammatory cascade (ω-6 PUFAs).[3] The products of two prominent ω-6 PUFAs, linoleic acid (LA; 18:2) and arachidonic acid (AA; 20:4), are detectable in many inflammatory diseases including sepsis.[3] These are acted on enzymatically—via lipoxygenase (LOX) or cyclooxygenase (COX) enzymes— or non-enzymatically to produce highly potent pro-inflammatory and pro-resolving signaling lipids.[3]

The dynamic nature of phospholipids and their potent downstream metabolites in sepsis have been explored in many contexts.[9–14] Mecatti et al.[9] investigated the changes in the phospholipid and FA composition via mass spectrometry in the plasma and erythrocytes of patients with sepsis and healthy volunteers, observing that lysophosphatidylcholines, sphingomyelins, and ω-3 PUFAs are decreased in patients with sepsis, whereas saturated and unsaturated phosphatidylcholines were upregulated. Another study specifically looked at the AA metabolites in the plasma of severely septic or healthy volunteers and found that two metabolites of

AA via cyclooxygenase (11-hydroxyeicosatetraenoic [11-HETE] and prostaglandin E2 [PGE2]) were significantly lower in patients with sepsis.[14] Furthermore, some have demonstrated a correlation between AA metabolites (leukotriene B4 [LTB4] thromboxane B2 [TXB2]) and platelet-activating factor acetylhydrolase, in the plasma of patients with sepsis.[12] In fact, the potential use of AA metabolites, specifically hydroxyeicosatetraenoic acid (20-HETE), as therapeutic agents against septic injury have been studied.[13]

Although prior studies of the changes in the lipidome of patients with sepsis have been explored, there were many limitations, namely: (1) small patient sample sizes, (2) lipidomic changes by sepsis severity were not reported, and (3) targeted analyses of pro-inflammatory and pro-resolving signaling lipid species have not been studied. Our study addresses these limitations by investigating the changes in the lipidome of 283 patients with sepsis of varying severity—rapid recovery versus CCI or early death—and compare them with healthy controls. The lipidomic data we generated from these patients not only allowed us to investigate major changes in 17 lipid classes but also enabled us to gain extensive resolution on the hundreds of respective subspecies that include specific FA tail composition within each lipid class, including: cholesterol esters (CEs), diacylglycerols, lactosylceramides (LacCers), phosphatidic acids (PAs), phosphatidylglycerols (PGs), sphingomyelin (SM), ceramide d18:0 (Cer d18:0), free FAs (FFAs), lysophosphatidylcholines (LPCs), phosphatidylcholines (PCs), phosphatidylinositol (PI), triglycerides (TGs), ceramide d18:1 (Cer d18:1), hexosylceramides (HexCER), lysophosphatidylethanolamine, phosphatidylethanolamine, and phosphatidylserine (PS). Subsequently, we used our recently developed liquid chromatography tandem mass spectrometry (LC–MS/MS) method that measures over 40 potent lipoxygenase and cyclooxygenase derived pro-inflammatory, pro-resolving, and anti-inflammatory bioactive lipid mediators, including those derived from ω-3 PUFAs— resolvin D1 (RVD1) and 4(S)-hydroxy docosahexaenoic acid (14S-HDHA)—and ω-6 PUFAs—9-, 13-hydroxyoctadecadienoic acid (HODE), and 5-, 11-, 12-, and 15-hydroxyeicosatetraenoic (HETE). We hypothesized that pro-inflammatory and pro-resolving signaling molecules derived from AA would be elevated in patients with CCI or early death—who were grouped together as the poor outcome group—versus the rapid recovery patients. Identifying specific signaling lipids that are elevated in poor outcome patients with sepsis may allow for future targeting and modulation of specific lipid enzymatic pathways to improve sepsis outcomes.

MATERIALS AND METHODS

Patient enrollment

Sepsis-3 criteria were utilized by trained research coordinators or providers to prospectively evaluate for the presence of sepsis in patients presenting to the UF Health Jacksonville emergency department within the first 24 h.[1] Patients were enrolled 7 days a week, between 8 a.m. and 10 p.m., into one of three observational studies and one clinical trial (LIPIDS-P), with comparable enrollment criteria.[6,15] Baseline blood samples of patients enrolled in the clinical trial were obtained prior to any drug administration. Patients admitted to the emergency department with minor non-infectious complaints and presenting normal vital signs were used as healthy controls. However, if control patients were excluded from participation if they had any of the following 1 week prior to enrollment: respiratory issues, abdominal discomfort, bleeding, suspicion of viral/bacterial infection, fever, or antibiotic use in the prior 2 weeks. All studies were approved by the University of Florida Institutional Review Board (IRB-01) under protocol numbers IRB201903081, IRB201601987, IRB201701349, and IRB201800027.

Data collection

Trained research coordinators reviewed and entered all clinical and laboratory data into a Research Electronic Data Capture (REDCap) database.[16,17] Data collected included comorbidities, residence, demographic information, and source of infection. Clinical variables included triage and enrollment vital signs, volume of intravenous fluids administered, antibiotic timing and duration, initial sequential organ failure assessment (SOFA) score, mechanical ventilation use and duration, and vasopressor use and duration. Hospital and ICU length of stay and mortality were also recorded.

Clinical outcomes and adjudication

The primary outcome was one of three categories: (1) early death (within 2 weeks of sepsis onset), (2) CCI (total ICU stay greater than 14 days with organ dysfunction or total ICU stay less than or equal to 14 days but discharged to long-term acute care, another hospital, or hospice), or (3) rapid recovery (all others).[18] Twenty-eight day mortality was also documented. During sepsis adjudication meetings, a minimum of two

clinician-investigators adjudicated the sepsis diagnosis, primary outcomes, primary and secondary source of infection, culture positivity, and hospital disposition.[19] In the event of any disagreement, a third clinician-investigator was included. Mortality for patients lost to follow-up was established by utilizing the social security death index.

Blood sampling and cholesterol measurement

Blood was collected at the time of patient enrollment and within 24 h of sepsis recognition and/or before initiation of any clinical trial drugs. Cholesterol levels and SOFA score measures including platelets, creatinine, and total bilirubin levels were measured via the hospital clinical laboratory. LDL-C was assessed by utilizing the Friedewald formula.[20]

Lipidomics analysis methods

Shotgun lipidomics

The extraction of lipids for shotgun lipidomic analysis has been described elsewhere.[21] Briefly, 25 μL of plasma was pipetted into a glass tube for extraction and modified Bligh and Dyer extraction was carried out with an initial ratio of 0.9:2:1 (water:methanol:chloroform) and a final ratio of 1.9:2:1.9. Prior to biphasic extraction, an internal standard mixture consisting of 70 lipid standards across 17 subclasses was added to each sample (AB Sciex 5040156, Avanti 330827, Avanti 330830, Avanti 330828, and Avanti 791642). Following two successive extractions, pooled organic layers were dried down in a Thermo SpeedVac SPD300DDA using ramp setting 4 at 35°C for 45 min with a total run time of 90 min. Lipid samples were resuspended in 300 μL of 1:1 methanol/dichloromethane with 10 mM ammonium acetate and transferred to robovials (Thermo 10800107) for analysis.

Samples were analyzed on the Sciex 5500 with DMS device (comparable hardware to the Sciex Lipidyzer Platform) with an expanded targeted acquisition list consisting of 1450 lipid species across 17 subclasses. Differential Mobility Device was tuned with EquiSPLASH LIPIDOMIX Avanti 330731 (Alabaster, AL). The instrument method including settings, tuning protocol, Multiple reaction monitoring (MRM) list, and data analysis workflow were performed as previously described.[22] Briefly, all tuning and data acquisition was carried out in Analyst 1.7.1 and data analysis was carried out using the Shotgun Lipidomics Assistant application. Compensation voltages (COV) tuning for individual lipid classes were selected by ramping the compensation voltage while acquiring representative class standards. These values are used to update the acquisition method. The 1450 targeted lipid species were acquired over two infusions of 75 μL. Each targeted MRM was acquired 20 times. Averaged raw signal was quantified against assigned standards and normalized to milliliters of plasma.

Lipid panel

The LC–MS/MS method utilized for the lipid panel was previously described.[23] Briefly, the lipid panel method we used covers 39 distinct bioactive lipids, degradation products, and pathway markers of the cyclooxygenase and the lipoxygenase products derived from AA, LA, docosahexaenoic acid, or eicosapentaenoic acid. We assigned each analyte to one of 19 distinct structurally identical or class-specific deuterated internal standards.[23] If an analyte lacked a structurally identical internal standard, we assigned an internal standard that shared the same basic structure and co-eluted within 0.5 min of the analyte in question.

Lipids were extracted from 100 μL of plasma by mixing BHT (20 μM), 50 μL of 20 ng/mL internal standard, and 150 μL of methanol. The sample was vortexed and spun down at 15,000 rpm for 10 min at room temperature. The supernatant was then collected and 1.8 mL of HCl-acidified H_2O (pH = 3–4) was added. Solid phase extraction was used with 3 cc Oasis HLB cartridges. The samples were then eluted with 2 mL of methanol, dried down under argon, and reconstituted in 100 μL of methanol for LC-MS analysis. Chromatography was performed on an Agilent 1290 UHPLC system using a Phenomenex Kinetex C18 column (2.6 μM particle size, 2.1 mm ID × 150 mm) and gradient elution. Solvent A was 75/25/0.1 (H_2O/acetonitrile/formic acid) and solvent B was 100/0.1 (acetonitrile/formic acid), and the gradient was as follows: 0–8.5 min, 0–85% B; 8.5–9.5 min, 85–100% B; 9.5–10.5 min, 100% B; 10.5–12 min, 100–0% B; 12–14 min, 0% B; with an additional 1 min equilibration at the starting conditions prior to each run. The column oven was set to 40°C, while the flow rate was 350 μL/min. Then, 5 μL of sample was injected for each run.

Mass spectrographic analysis was performed on a SCIEX 5500 QTrap run in negative ion mode and controlled by Analyst 1.6.2 software. Two separate MRM transitions were determined for each compound, and DP, EP, CE, and CXP for each compound were all manually optimized by tuning on direct infusions of ~50 ng compound/mL 50% B.

Data analysis was performed using MultiQuant software, with the concentration of each analyte being determined relative to its internal standard and against a standard curve with concentrations from 500 ng/mL to 100 pg/mL. Final data was normalized to plasma volume.

Statistical analysis

Statistical analysis was performed using R version 4.3.0 (R Core Team 2023, Vienna, Austria). Our analysis involved two types of data: Shotgun Lipidomics and lipid panel data. Due to differences in sample sizes and data structures, the comparison protocols for these two datasets were similar but not identical.

Shotgun lipidomics

We filtered out lipids with missing values, resulting in 354 lipids for the subsequent analysis. To address the issue of unbalanced outcome groups, where there were 192 samples in the rapid recovery group and 82 in the CCI/early death group, we randomly selected 82 samples from the rapid recovery group 100 times. For each balanced dataset, we performed *t*-tests on each lipid to detect pairwise differences between the rapid recovery group and the CCI/early death group. To account for multiple comparisons, we applied a false discovery rate (FDR) correction using the Benjamini–Hochberg method across all lipids, yielding adjusted *p* values.[24] Subsequently, we used the Cauchy combination rule to aggregate the adjusted *p* values across 100 resampled datasets into overall adjusted *p* values.[25] Lipids with overall adjusted *p* values less than 0.05 were considered statistically significant. It is important to note that the presented boxplots illustrating differences in lipid levels by outcome were based on the original data without resampling, whereas adjusted *p* values were derived from the statistical comparisons.

Lipid panel

For the lipid panel analysis, we initially excluded lipids with more than 25% missing values within each group (rapid recovery, CCI, or early death). This filtering left us with eight lipids that were measured in at least 75% of samples for each group, with five of these lipids containing no missing values. To address the issue of missing data, we used the mice package in R to perform multiple imputations using predictive mean matching.[26] This process resulted in the generation of five imputed datasets, each with 10 iterations. Due to the unbalanced nature of the outcome groups (189 samples in the rapid recovery group and 73 in the CCI/early death group), we randomly selected 73 samples from the rapid recovery group for each imputed dataset 100 times. Similar to the Shotgun Lipidomic analysis, for each balanced dataset we conducted *t*-tests for each lipid to identify pairwise differences between the rapid recovery group and the CCI/early death group. We also utilized FDR correction using the Benjamini–Hochberg method across all lipids to calculate adjusted *p* values.[24] Subsequently, we applied the Cauchy combination rule to aggregate the adjusted *p* values across 500 balanced datasets into overall adjusted *p* values.[25] Lipids with overall adjusted *p* values less than 0.05 were considered statistically significant. Notably, the presented boxplots depicting differences in lipid levels by outcome were based on the original data without imputation and resampling, whereas adjusted *p* values were derived from the statistical comparisons.

RESULTS

Clinical descriptors

There were 283 patients with sepsis included in this study with clinical features presented in Table 1. The median age was 63 years, and 53% of patients were men. There were slightly more African American patients (51%) than White patients (47%), or other races or ethnicities (2%). Age, gender, and race or ethnicity were similar between the rapid recovery and the CCI/early death groups. The prevalence of septic shock within the overall cohort was 53%, but higher in the CCI/early death group (74%) than the rapid recovery group (44%). Median SOFA scores were also higher for CCI/early death (10, interquartile range [IQR]: 7–12) compared to rapid recovery (6, IQR: 4–9). Rates of in-hospital and 28-day mortality were much higher in the CCI/early death group at 27% and 69%, respectively. In-hospital and 28-day mortality rates were 0% and 2%, respectively, for the rapid recovery group. Sources of infections were similar, although the CCI/early death group had more pulmonary infections. Comorbidities were also similar between groups.

Lipidomic screen of lipid classes in sepsis outcome-based analysis

The study included 317 samples, including 39 healthy control volunteers and 274 patients with sepsis. Healthy control patients were included in the analysis to provide a healthy baseline lipidomic profile as a comparator for all patients with sepsis. Their demographics and chief complaints are presented in Table 1. Of the patients with sepsis, 192 were categorized as rapid recovery, 47 developed CCI, and 35 were early deaths, with the CCI and early death patients grouped together for analysis (Table 2). We initially investigated changes in various lipid classes, including PGs, CEs, LPCs, LacCers, FFAs, SM, TGs, Cer d18:1, PAs, PS, HexCERs, PIs, and PCs (Figure 1).

TABLE 1 Descriptive statistics.

Variable	Total cohort ($n = 283$)	Rapid recovery ($n = 199$)	CCI/early death ($n = 84$)	Controls ($n = 39$)
Age (median [IQR])	63.00 [56.00, 72.79]	61.92 [55.00, 71.00]	65.44 [58.49, 74.37]	60.0 [55.5, 67.0]
Gender – male (*n*, %)	150 (53%)	105 (53%)	45 (54%)	20 (51%)
Race (*n*, %)				
White	132 (47%)	91 (46%)	41 (49%)	19 (49%)
African American	145 (51%)	103 (52%)	42 (50%)	20 (51%)
Other	6 (2%)	5 (2%)	1 (1%)	–
Septic shock (*n*, %)	150 (53%)	88 (44%)	62 (74%)	NA
SOFA score (median [IQR])	7 [4, 10]	6 [4, 9]	10 [7, 12]	NA
In hospital death (*n*, %)	23 (8%)	0 (0%)	23 (27%)	NA
28 days mortality (*n*, %)	62 (22%)	4 (2%)	58 (69%)	NA
Infection source (*n*, %)				
Urinary tract	100 (35%)	73 (37%)	27 (32%)	NA
Pulmonary	104 (39%)	63 (32%)	41 (49%)	NA
Skin/soft tissue	38 (13%)	29 (15%)	9 (11%)	NA
Intra-abdominal	31 (11%)	22 (11%)	9 (11%)	NA
Blood without another source	32 (11%)	24 (12%)	8 (10%)	NA
Unknown	39 (14%)	33 (17%)	6 (7%)	NA
Other	16 (6%)	10 (5%)	6 (7%)	NA
Comorbidities (*n*, %)				
Diabetes	118 (42%)	89 (45%)	29 (35%)	NA
Chronic obstructive pulmonary disease	52 (18%)	36 (18%)	16 (19%)	NA
End-stage renal disease	52 (18%)	37 (19%)	15 (18%)	NA
Active cancer	29 (10%)	16 (8%)	13 (15%)	NA
Human immunodeficiency virus infection	6 (2%)	5 (3%)	1 (1%)	NA
Cholesterol levels (median [IQR])				
Total cholesterol	94.0 [74.0, 122.0] (6 missing)	96.6 [75.0, 125.5] (3 missing)	84.0 [71.0, 120.0] (3 missing)	181.0 [146.0, 215.5]
HDL-C	26.0 [16.0, 38.0] (4 missing)	28.0 [16.0, 38.0] (2 missing)	24.5 [11.5, 38.5] (2 missing)	52.3 [39.6, 69.3]
LDL-C	41.0 [27.0, 61.5] (12 missing)	42.3 [28.2, 62.0] (5 missing)	36.0 [24.0, 58.7] (7 missing)	100.2 [76.3, 128.0]
Triglycerides	112.0 [78.0, 151.0] (6 missing)	114.0 [84.7, 149.5] (3 missing)	99.0 [72.0, 153.0] (3 missing)	100.0 [70.6, 139.0]

Note: Comparison of patient demographics, organ failure severity, infectious source, and comorbidities by rapid recovery versus chronic critical illness (CCI)/early death categories. Control patient demographic data are listed. Several data elements are not applicable (NA) to controls.

Abbreviations: CCI, chronic critical illness; IQR, interquartile range; SOFA, sequential organ failure assessment score.

Although no significant changes were observed in any lipid classes in rapid recovery versus CCI/early death (Table S1, Figure S1), PG levels were lower, whereas CE and LPC levels were elevated in rapid recovery compared to CCI/early death (Figure 1). Only three lipids were significantly differentially abundant between groups after multiple hypothesis testing correction (Table 2). FA 12:0 was significantly decreased in the CCI/early death group when compared to the rapid recovery group (Figure 2c). However, FA 17:0 and 20:1 were significantly elevated in the CCI/early death group when compared to the rapid recovery group (Figure 2a,b). We also compared changes in the lipidome by African American versus White race (Figure S2).

TABLE 2 Lipidyzer lipidomic screen comparison.

Lipid	Total cohort ($n = 274$)	Rapid recovery ($n = 192$)	CCI/early death ($n = 82$)	Adjusted p value[a]
FA 17:0 (median [IQR])	10.54 [9.17, 11.54]	10.25 [8.46, 11.44]	11.06 [10.22, 11.74]	0.00
FA 20:1 (median [IQR])	4.09 [3.41, 4.77]	3.97 [3.33, 4.57]	4.38 [3.79, 5.17]	0.02
FA 12:0 (median [IQR])	3.14 [2.15, 4.80]	3.34 [2.29, 5.68]	2.74 [1.95, 3.79]	0.03

Note: Results of Lipidyzer lipidomic screen by rapid recovery versus CCI/early death patients. FAs 17:0 and 20:1 were elevated in CCI/early death, and FA 12:0 was decreased in CCI/early death compared to rapid recovery.

Abbreviations: CCI, chronic critical illness; FA, fatty acid; IQR, interquartile range.

[a]Reported p values are based on 100 resampling iterations, followed by false discovery rate correction for multiple comparisons.

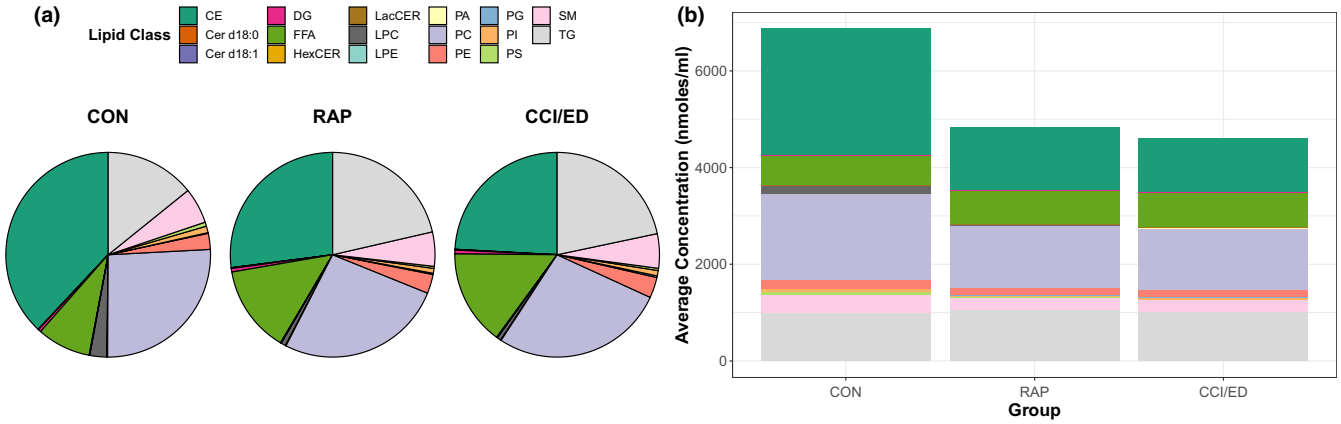

FIGURE 1 Lipidomic changes in sepsis outcome-based analysis: The lipidome was evaluated from the serum of healthy, control patients (CON) and patients with sepsis that were categorized by sepsis severity—Rapid Recovery (RAP) or Chronic Critical Illness and Early Death (CCI/ED). The concentration of the lipid classes is reported as average concentration in nmoles/mL. The levels of each lipid class are reflected as a pie chart (a) or bar graph (b) within each sample group— CON, RAP, and CCI/ED. The lipid classes included are cholesterol esters (CEs), diacylglycerols (DGs), lactosylceramides (LacCers), phosphatidic acids (PAs), phosphatidylglycerols (PGs), sphingomyelin (SM), ceramide d18:0 (Cer d18:0), free fatty acids (FFAs), lysophosphatidylcholines (LPCs), phosphatidylcholines (PCs), phosphatidylinositol (PI), triglycerides (TGs), ceramide d18:1 (Cer d18:1), hexosylceramides (HexCERs), lysophosphatidylethanolamine (LPE), phosphatidylethanolamine (PE), and phosphatidylserine (PS).

Alterations in pro-inflammatory and pro-resolving signaling lipids in sepsis outcome-based analysis

To interrogate changes in the pro-inflammatory and pro-resolving signaling lipids in our lipidomic screen, we utilized the same patient pool as in the Shotgun Lipidomic analysis. For this analysis, 262 patients with sepsis samples were included, with 189 rapid recovery, 45 CCI, and 28 early death (Table 3). As with the lipidomic analysis, the CCI and early death groups were combined for analysis as in prior studies.[27] We detected many pro-inflammatory signaling lipids from ω-6 PUFAs, including 9-,13-HODE and 5-,11-, 12-, and 15-hydroxyeicosatetraenoic (HETE), along with pro-resolving lipid mediators 14(S)-hydroxy docosahexaenoic acid (14S-HDHA) and Resolving D1 (RVD1) derived from ω-3 PUFAs (Table 3). There were multiple analytes significantly increased in this analysis (Table 3). Four lipids were all significantly elevated in the CCI/early death group when compared with the rapid recovery group, including 12-HETE, 15-HETE, 11-HETE, and 14S-HDHA (Figure 3a–d, respectively). These potent signaling lipids are all oxidation products derived from AA and can be formed enzymatically or non-enzymatically.[28] More specifically, the enzymatic reactions are facilitated by various lipoxygenases including 12,15-lipoxygenase (12,15-LOX) for 12-HETE and 15-HETE.[28] 11-HETE can be generated non-enzymatically via an auto-oxidation reaction or by COX.[28] Interestingly, 14S-HDHA can be generated via 12-LOX or by 15-LOX processing of docosahexaenoic acid.[29]

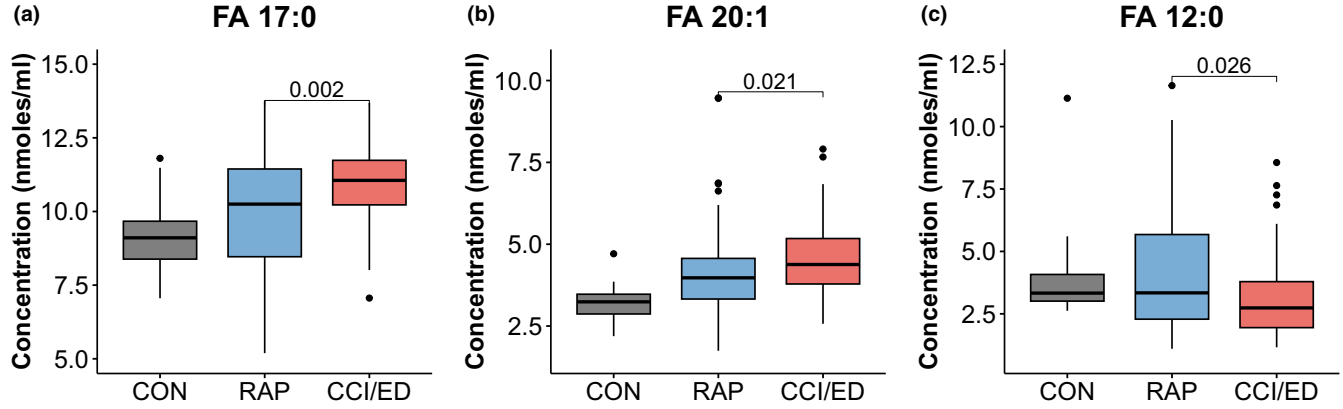

FIGURE 2 Significant changes in the lipidome in sepsis outcome-based analysis: Free fatty acid (FFA) 17:0 (a), 20:1 (b) and 12:0 (c) were the only three significantly altered lipids in the lipidomic screen between Rapid Recovery (RAP) or Chronic Critical Illness and Early Death (CCI/ED). The concentration is reported in nmoles/mL. Reported *p* values are based on 100 resampling iterations, followed by false discovery rate correction for multiple comparisons.

TABLE 3 Pro-inflammatory and pro-resolving signaling lipid panel comparison.

Lipid	Total cohort ($n=262$)	Rapid recovery ($n=189$)	CCI/early death ($n=73$)	Adjusted *p* value[a]
Pro-inflammatory signaling lipids				
15-HETE (median [IQR])	0.20 [0.14, 0.37]	0.19 [0.13, 0.32]	0.24 [0.17, 0.54]	0.03
12-HETE (median [IQR])	6.99 [1.91, 21.15]	5.82 [1.69, 16.33]	9.00 [3.03, 45.54]	0.04
11-HETE[b] (median [IQR])	0.12 [0.06, 0.23] ($n=245$)	0.11 [0.06, 0.21] ($n=175$)	0.17 [0.10, 0.38] ($n=70$)	0.04
13-HODE (median [IQR])	2.85 [2.09, 4.21]	2.89 [2.12, 4.11]	2.80 [2.04, 4.33]	0.88
9-HODE (median [IQR])	1.73 [1.20, 2.55]	1.70 [1.18, 2.53]	1.78 [1.29, 2.62]	0.92
5-HETE (median [IQR])	0.32 [0.18, 0.56]	0.31 [0.17, 0.55]	0.34 [0.19, 0.56]	0.97
Pre-resolving signaling lipids				
14S-HDHA[b] (median [IQR])	0.46 [0.17, 0.89] ($n=236$)	0.36 [0.13, 0.77] ($n=174$)	0.72 [0.36, 1.66] ($n=62$)	0.02
RVD1[b] (median [IQR])	0.06 [0.04, 0.11] ($n=254$)	0.06 [0.04, 0.11] ($n=182$)	0.06 [0.04, 0.11] ($n=72$)	0.92

Note: Results of pro-inflammatory and pro-resolving signaling lipid panel by rapid recovery versus CCI/early death patients. 12-HETE, 15-HETE, 11-HETE, and 14S-HDHA were elevated in CCI/early death compared to rapid recovery.

Abbreviations: CCI, chronic critical illness; IQR, interquartile range.

[a]Reported *p* values are based on 100 resampling iterations and subsequent multiple imputations, followed by false discovery rate correction for multiple comparisons.

[b]Lipids with missing values.

DISCUSSION

This study allowed for a powerful and robust exploration of lipidomic changes on a class, species, and downstream signaling lipid level in this novel characterization of sepsis outcome. There was an abundance of lipid species identified, although only three FFAs (17:0, 12:0, and 20:1) were significantly altered between the rapid recovery and

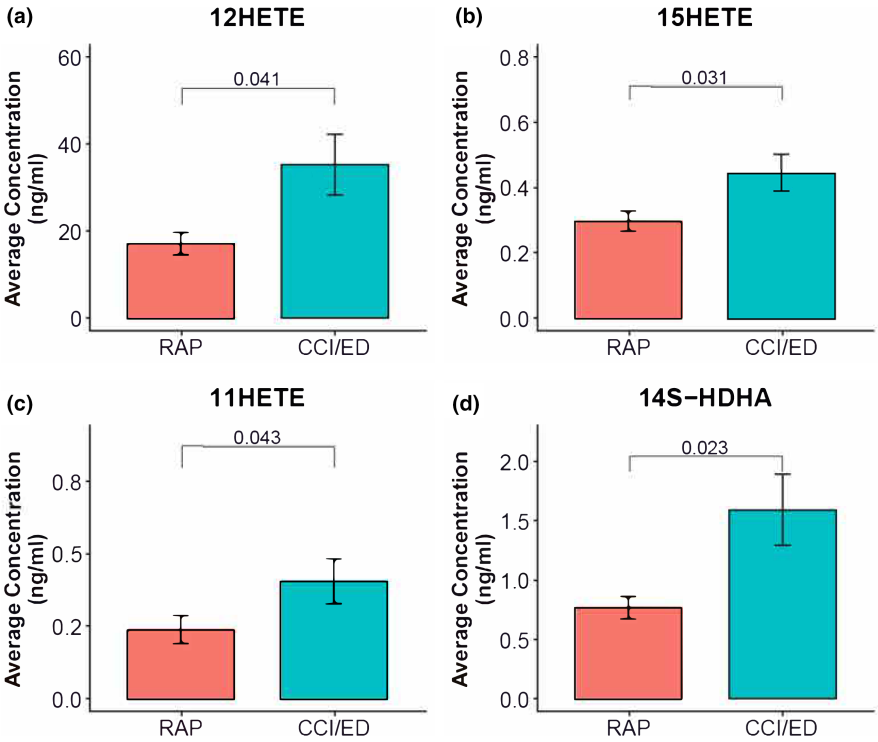

FIGURE 3 Significant changes in the potent signaling lipids in sepsis outcome-based analysis. Four potent pro-inflammatory, pro-resolving signaling lipids were significantly increased in Chronic Critical Illness and Early Death (CCI/ED) versus Rapid Recovery (RAP). The average concentration is reported in ng/mL. Data are represented mean ± SEM. Reported *p* values are based on 100 resampling iterations and subsequent multiple imputations, followed by false discovery rate correction for multiple comparisons.

CCI/early death groups. Furthermore, there were four significantly altered proinflammatory and pro-resolving signaling lipids—12-HETE, 15-HETE, 11-HETE, and 14S-HDHA—changes observed in the sepsis outcome-based analysis. Our study findings support our hypothesis, that pro-inflammatory and pro-resolving signaling molecules derived from AA would be elevated in poor outcome patients with sepsis compared to those who have a more sustained and rapid recovery.

Many studies have found an increase in plasma FAs in sepsis.[3,30,31] In mouse cecal ligation and puncture models of sepsis, there is an increased abundance in plasma FFAs due to decreased liver PPAR-α levels.[31] In addition, increased plasma abundance FFA were found in non-surviving patients with sepsis, particularly elevated levels of saturated FFA.[30] In our sepsis outcome-based analysis, FA 17:0 and FA 20:1 were significantly elevated in the CCI/early death group compared to the rapid recovery group, with the opposite observed for FA 12:0. It is well-established that cholesterol levels sharply decline in patients with sepsis.[15] Interestingly, FA 12:0, also known as Lauric acid, has been shown to increase circulating cholesterol levels.[32] Our data suggest that the decrease in FA 12:0 in the CCI/early death group could partially explain the decreased cholesterol levels observed in sepsis by numerous prior studies.[33–35]

The pro-inflammatory nature of sepsis—particularly enhanced by leukocytes recruited to the sites of infection and inflammation—results in the upregulation of pro-inflammatory AA metabolites, or eicosanoids, including the four lipids (12-HETE, 15-HETE, 11-HETE, and 14S-HDHA) that were all significantly elevated in the CCI/early death group when compared with the rapid recovery group.[3] The 12-HETE, along with thromboxane B2, has specifically been shown to increase during sepsis to drive thrombus generation by promoting platelet activation and aggregation, along with vasoconstriction. Although a previous study observed that 11-HETE was reduced in patients with sepsis, many other studies have demonstrated the pro-inflammatory nature of 11-HETE in various disease models including obesity and atherosclerosis.[14,36,37] The 15-HETE has also been implicated as a pro-inflammatory lipid in various disease models, including cardiovascular disease and pulmonary hypertension.[38–40] Of note, 15-HETE was found in the heart of patients with ischemic heart disease and it was also observed to increase blood clot formation.[38] Furthermore, our group recently reported that oral treatment of 15-HETE in the diet was sufficient to induce pulmonary hypertension in mice, which was ameliorated by the treatment of transgenic 6F, an apoA-1 mimetic, that is known to prevent the formation and aggregation of oxidized lipids.[40] Interestingly, the elevation of 14S-HDHA, an ω-3 PUFA-derived pro-resolving mediator, in patients with CCI/early death may indicate an endogenous attempt at regulating inflammation in poor outcome patients with sepsis. It is also the basis and rationale for the potential use of statins in sepsis.

This study identified novel pro-inflammatory and pro-resolving signaling lipids that have not been

thoroughly investigated in the context of sepsis, namely 15-HETE and 14S-HDHA. As briefly mentioned, both of these lipids are enzymatically regulated by 12,15-LOX.[28] A recent study demonstrated the omega-3 lipid rich emulsions aid in the resolution of inflammation and sepsis survival in a sterile peritonitis and murine polymicrobial sepsis model, which was in part dependent on 5-LOX and 12,15-LOX.[41] Similarly, Hall et al.[42] showed that administration of a 10% intravenous fish oil lipid emulsion to patients with sepsis in an ICU setting improved organ failure compared to control patients. Therefore, targeting or modulating 12,15-LOX activity may serve as a potentially important therapeutic strategy against sepsis.

This study had two main limitations. First, there was an imbalance in the number of patients in the rapid recovery versus the CCI and early death groups. However, we used resampling to ensure that we were able to statistically control this imbalance. Second, there were lipids that we were unable to completely detect, and subsequently did not analyze, in both groups. This being a translational study, there is always the potential for loss of lipids during processing or during storage due to oxidation. However, we carefully process and store all samples for lipid analysis during and after enrollment to minimize these potential issues, which are to some degree unavoidable. In addition, our approach limited the use of imputation methods to only the few lipids that were minimally missing, and therefore allowed us to be sure that we were measuring endogenously circulating and active lipids. We believe this provided a more valid approach to truly understanding lipid metabolism in sepsis.

CONCLUSION

In conclusion, we identified three FFAs and four significantly altered pro-inflammatory and pro-resolving signaling lipids (12-HETE, 15-HETE, 11-HETE, and 14S-HDHA) of the AA pathway that were significantly altered between the rapid recovery and CCI/early death groups. Future studies targeting the 12/15-LOX pathway of AA metabolism may be important to improving sepsis outcomes in a subset of patients.

AUTHOR CONTRIBUTIONS

D.S. and F.W.G. wrote the manuscript. D.S., D.W., L.P.B., K.G., S.D., S.T.R., and F.W.G. designed the research. D.S., D.W., K.J.W., S.T.R., and F.W.G performed the research. D.S., D.W., K.J.W., K.G., and S.D. analyzed the data. K.J.W. contributed new reagents/analytical tools.

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CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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