

A Reproducible Ceramide Phenotype of Sepsis Across Aetiologies – A Monocenter Cohort Study

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Purpose: Sepsis due to SARS-CoV-2 shares features with non-COVID-19 sepsis. Ceramides—bioactive sphingolipids—exist as long-chain (LC) and very-long-chain (VLC) species with opposing signaling effects. Whether LC and VLC ceramides are altered similarly in COVID-19 vs non-COVID-19 sepsis, and the therapeutic relevance of sphingomyelinase inhibition, remains uncertain. We characterised circulating ceramides in patients with systemic inflammatory response syndrome (SIRS) or sepsis with and without SARS-CoV-2.

Patients and Methods: We performed targeted lipidomics on serum/plasma in two cohorts: (i) plasma from 157 SIRS/sepsis patients with different disease etiologies, including 23 patients with SARS-CoV-2 and 23 healthy controls; (ii) serum from 96 SIRS/sepsis patients with SARS-CoV-2 and 17 patients without SIRS/sepsis and without SARS-CoV-2. We quantified individual ceramide species in the serum or plasma of patients and controls simultaneously. The ceramide species levels of patients and controls, as well as patients with and without SARS-CoV-2, were compared. In the patients we assessed associations with C-reactive protein, cholesterol, and survival.

Results: Relative to non-septic comparators, SIRS/sepsis showed higher ceramide 18:1;O2/16:0, 18:0, 20:0, and 24:1 ($p < 0.001$ for all), and lower ceramide 18:1;O2/23:0, 24:0 ($p = 0.001$ for both), and 26:0 ($p < 0.001$). Ceramide profiles were comparable between SARS-CoV-2 and non-SARS-CoV-2 sepsis ($p > 0.05$). Several ceramides correlated positively with C-reactive protein and cholesterol, yet these variables did not account for the divergent regulation of LC (increased) versus VLC (mostly decreased) species. Neither individual ceramides nor the LC/VLC ratio was associated with survival ($p > 0.05$).

Conclusion: Sepsis is characterised by a reproducible ceramide signature that does not differ by SARS-CoV-2 status. These data argue against non-selective acid sphingomyelinase inhibition and instead support evaluation of selective ceramide synthase targeting as a therapeutic approach in sepsis.

Keywords: sepsis, SIRS, SARS-CoV-2, COVID-19, ceramide, sphingolipids, lipidomics, acid sphingomyelinase, ceramide synthase, biomarkers, critical care

Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection.^{1,2} SARS-CoV-2 can precipitate sepsis that mirrors many—though not all—features of sepsis from other aetiologies.^{3,4} The outcome of sepsis is influenced by a complex interplay between anti-inflammatory and pro-inflammatory responses within the body. Consequently, efforts aimed at controlling only pro-inflammatory pathways have proven to be ineffective.⁵ It is evident that lipids play a pivotal role in both the pro-inflammatory and anti-inflammatory responses of the body.⁶ Hypocholesterolemia is a defining feature of sepsis independent from disease aetiology, with decreased levels of both low-density lipoprotein (LDL) and high-density lipoprotein (HDL).^{7,8} Ceramides are bioactive sphingolipids that increase during inflammation and are components of lipoproteins.^{9,10} In humans, experimental endotoxaemia elevates ceramide concentrations in plasma in very-low-density lipoprotein (VLDL) and LDL, but not HDL.¹¹ Acid sphingomyelinase

(ASM) produces ceramides, and its systemic levels rise alongside disease severity in patients with sepsis.¹² Accordingly, circulating ceramides have also been explored as diagnostic and prognostic biomarkers across inflammatory conditions.^{13–16} However, it remains unclear whether the circulating ceramide lipidome differs between patients with sepsis who have or do not have SARS-CoV-2 infection.

Among intensive care unit patients with SARS-CoV-2 infection, serum concentrations of six ceramide species were higher than in healthy controls.¹³ Ceramide bioactivity varies by acyl-chain length: long-chain (LC; C16–C20) species generally promote insulin resistance and cell death, whereas very-long-chain (VLC; C22–C26) species often exert opposing effects.¹⁷ Notably, both LC and VLC ceramides were elevated in COVID-19 serum.¹³ Other cohorts report higher plasma VLC ceramides in SARS-CoV-2 infection, with further increases in severe disease,¹⁸ and higher C16:0, C18:0, C22:0, and C24:1 ceramide levels in severe/critical versus moderate illness; ceramide C24:0 was unchanged, and ceramide C23:0 and C26:0 were not assessed.¹⁹ In mechanically ventilated COVID-19 patients, plasma ceramide C16:0 was ~threefold higher, with experimental evidence implicating this species in vascular injury; in that study, VLC ceramide C22:0, C24:0, and C26:0 were comparable to controls.²⁰

A 2002 study, which for obvious reasons has not included patients with SARS-CoV-2, showed that sepsis is associated with increased levels of ceramides C16:0, C18:0, C20:0, C22:1 and C24:1, and decreased levels of ceramides C23:0 and C24:0, compared to controls.¹⁴ While some studies have reported higher total ceramide levels in the blood of patients with sepsis, others have found similar levels in sepsis patients and healthy controls.^{14,21} Total ceramide levels were higher still in patients with sepsis than in patients with systemic inflammatory response syndrome (SIRS), who were also hospitalized in the intensive care unit.²¹

Circulating ceramides are transported predominantly on lipoproteins,¹¹ which also carry cholesterol.²² We recently showed that patients with SARS-CoV-2–related sepsis have higher plasma cholesteryl ester levels than those with sepsis from other pathogens.²³ Analogously, ceramide species may differ by sepsis aetiology, with distinct patterns reported in SARS-CoV-2 versus non-COVID sepsis.^{14,21} The current gap in the research is that the ceramide profiles of critically ill patients with and without a SARS-CoV-2 infection have not yet been directly compared.

Despite decades of extensive experimental and clinical research, our ability to positively influence the course and outcome of sepsis remains limited.² Observational clinical data suggest that antidepressants with high ASM-inhibitory activity (FIASMA), such as fluoxetine, may be associated with lower mortality rates from COVID-19.^{24,25} In rats with systemic inflammation, fluoxetine reduced cytokine levels and blunted hypothermia.²⁶ Desipramine, another FIASMA, protected mice from liver damage during the acute phase.²⁷ These studies suggest that reducing ASM activity and, consequently, ceramide levels is a promising approach to treating sepsis.

Interpretation of blood lipid profiles should account for liver cirrhosis, which is characterised by hypolipoproteinaemia.^{17,28} In cirrhosis, most serum ceramide species are reduced, with progressive decreases in both LC and VLC species as disease severity increases.^{29,30}

The application of targeted and nontargeted lipidomics reveals distinct advantages and limitations. Untargeted lipidomics is defined as an unbiased approach that is aimed at identifying and quantifying a wide range of lipid species.³¹ This exploratory technique does not specify the lipids of interest in advance, thereby facilitating the discovery of novel and unexpected lipid species. Conversely, targeted lipidomics constitutes an analytical approach for the precise quantification of specific lipid species through the use of internal standards.³¹ The present study employed targeted lipidomic analysis to investigate the levels of ceramide species in clinical samples.

Previous studies have not compared the ceramide lipidome of COVID-19 and non-COVID-19 sepsis patients.^{14,21} The serum lipidome of these patients differs,^{23,32} and given the central role of ceramides in inflammation, this analysis could have diagnostic and therapeutic implications.

The primary objective of this study was to ascertain whether there is a difference in the blood ceramide lipidome between patients with SARS-CoV-2 sepsis and patients with sepsis from other causes. Furthermore, the levels of ceramide species in controls and patients with sepsis were compared, and their associations with clinical markers of disease severity were evaluated.

Materials and Methods

Study Cohorts

Cohort 1

Between August 2018 and January 2024, plasma samples were taken from patients admitted to the medical intensive care unit at our hospital ([Figure S1](#)). Patients were categorized as having sepsis, septic shock, or systemic inflammatory response syndrome (SIRS) using the Sepsis-3 criteria³³ and the SIRS criteria.³⁴ Plasma samples from patients with SARS-CoV-2 infection were collected between October 2020 and January 2023. Participants with hepatitis virus, human immunodeficiency virus, or multidrug-resistant illnesses, as well as patients younger than 18 years, were excluded. All other patients who were willing to participate were included in this retrospective analysis. Patients who were discharged from the critical care unit alive were considered survivors, while those who passed away in the hospital were considered non-survivors.

The study included 23 healthy controls (10 males and 13 females). The median age of the controls was 42 years (range 25–78), and the controls were younger than the patients ($p < 0.001$). The study protocol was approved by the ethics committee of the University Hospital of Regensburg (protocol number 18–1029-101) and was conducted in accordance with the latest guidelines on good clinical practice and the latest Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study. This study cohort is described in [Table 1](#). The study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.³⁵ The completed STROBE checklist is included as a [Supplementary Material](#).

In Cohort 1, lysophosphatidylcholine species have already been analysed.³² Moreover, the plasma triglyceride and cholesterol levels of most of these patients were also determined and published recently.²³ Over time, the present cohort has grown, and additional protein biomarkers, such as IL-32, have been determined.³⁶ It is not feasible to cite all the papers that included patients enrolled in this study, as extensive self-citation is not advisable. The current study does not overlap with any previously published data.

Cohort 2

Serum samples of patients with a confirmed SARS-CoV-2 were collected between April 2020 and January 2024 during their hospital stays ([Figure S1](#)). All patients over 18 years who were willing to participate in the study were included. Of these patients, 37 exhibited symptoms indicating SIRS,^{34,37} and did not require intensive care. In contrast, 59 patients required intensive care admission due to sepsis/septic shock³³ ([Table S1](#)). It is important to note that patients with liver cirrhosis were excluded. This cohort does not overlap with the one described above (Cohort 1). The control group comprised the serum of 17 patients hospitalised for various diseases at the time that the serum of the patients with SARS-

Table 1 Characteristics of Patients with SIRS/Sepsis/Septic Shock, Excluding Those with Liver Cirrhosis. Data are Given as Median (Minimum–Maximum)

Parameters	SIRS/Sepsis/Septic Shock Patients
Males/Females	88/38
Age, years	60 (21–93)
Body mass index, kg/m ²	26.2 (15.4–55.6) ¹²⁴
SIRS/Sepsis/Septic shock	29/33/64
C-reactive protein, mg/L	183 (23–697)
Procalcitonin, ng/mL	1.11 (0.05–270.00) ¹²²
Interleukin-6, pg/mL	75 (0–5702) ¹²²
Total bilirubin, mg/dL	0.80 (0.10–23.90) ¹¹⁹
Albumin, g/L	23.0 (6.3–42.0) ¹¹⁶
Aspartate aminotransferase, U/L	40 (6–1597) ¹¹³
Alanine aminotransferase, U/L	32 (6–770) ¹¹¹
Gamma-glutamyl transferase, U/L	122 (11–1266) ¹⁰⁰

Note: The numbers in superscript indicate the number of patients for whom this data was available when the data collection did not cover the whole cohort.

CoV-2 was obtained. These patients had almost normal C-reactive protein levels and were not infected with SARS-CoV-2 ([Table S1](#)). The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University Hospital of Regensburg (protocol code 18–1029_2-101). Informed consent was obtained from all subjects involved in the study. The study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.³⁵ The completed STROBE checklist is included as a [Supplementary Material](#).

In Cohort 2, the analysis of serum cholesteryl ester species has already been conducted.³⁸ Additional protein biomarkers, such as S100A12, were determined.³⁹ Due to the extensive nature of self-citation, it is not feasible to cite all the papers in which patients who were also enrolled in this study were included. The current study does not overlap with any previously published data.

Laboratory data were provided by the Institute of Clinical Chemistry and Laboratory Medicine at the University Hospital of Regensburg.

Measurement of Plasma Ceramide Species

Lipids were extracted from 10 µL of plasma or serum according to the protocol of Bligh and Dyer.⁴⁰ Prior to lipid extraction, non-natural lipid species (ceramide 18:1;O2/17:0 and ceramide 18:1;O2[D7] /18:0, Avanti Polar Lipids, AL, USA) were added as internal standards. The dried chloroform phase was dissolved in a methanol/chloroform (3:1, v/v) mixture containing 7.5 mM ammonium acetate.⁴¹ Ceramide species were quantified by direct flow injection analysis (FIA) using a Waters Xevo TQ-S micro (Waters, Milford, Massachusetts, USA) triple quadrupole mass spectrometer (FIA-MS/MS) with an electrospray ionization in positive ion mode and a fragment ion of m/z 264. The instrument was operated with the following settings: Capillary voltage 3.7 kV; cone voltage 45 V; desolvation temperature 200 °C; desolvation and cone gas flow 700 and 75 L/hr, respectively. A collision energy of 25 eV was applied, and the selected reaction monitoring included both mass transitions for $[M+H]^+$ and $[M+H-H_2O]^+$, with a fragment ion of m/z 264 and an 8 ms dwell time (see [Table S2](#)). Mass spectra were acquired at a flow rate of 10 µL/min, with 40 spectra averaged and processed. Data were corrected for Type-II isotopic overlap and quantified by calibration curves according to the principles described previously.⁴² Analysis of plasma/serum cholesterol levels of these cohorts has been described.^{23,43}

Statistical Analysis

Data are displayed as box-and-whisker plots (median line; box = first to third quartile; whiskers extend to the most extreme values within 1.5×IQR; values beyond are plotted as outliers [circles] or extremes [asterisks]). Medians and ranges for each dataset are provided in the tables. Analyses were performed in IBM SPSS Statistics v26.0 (IBM Corp., 2019). Shapiro–Wilk tests indicated non-normality for all ceramide species (all $p < 0.001$). Accordingly, group comparisons used Kruskal–Wallis tests (for >2 groups) and Mann–Whitney U -tests (for two groups); categorical variables were compared with χ^2 -tests; associations between continuous variables were assessed using Spearman's ρ . Two-sided $p < 0.05$ was considered statistically significant.

Results

Ceramide Species in Patients with Liver Cirrhosis

The study included 157 patients, 31 of whom had liver cirrhosis. The levels of all ceramide species analyzed, which were ceramide 18:1;O2/16:0, 18:0, 20:0, 22:0, 23:0, 24:0, 24:1, and 26:0, decreased in cirrhosis ($p < 0.001$ for all but ceramide 18:1;O2/26:0 with $p = 0.023$). The concentrations in plasma were 65%, 26%, 30%, 36%, 43%, 46%, 42%, and 84% of those in non-cirrhosis patients, respectively. The LC/VLC ratio remained unchanged ($p = 0.679$).

Previous studies in patients with cardiovascular disease suggested that ceramide 18:1;O2/16:0, ceramide 18:1;O2/18:0, ceramide 18:1;O2/20:0 and ceramide 18:1;O2/24:1 levels relative to ceramide 18:1;O2/24:0 levels are of diagnostic value.^{44,45} The ratio ceramide 18:1;O2/16:0 to ceramide 18:1;O2/24:0 was increased in cirrhosis ($p = 0.001$) whereas ceramide 18:1;O2/18:0 and 20:0 to ceramide 18:1;O2/24:0 levels decreased ($p < 0.001$ for both). The ceramide 18:1;O2/24:1 to ceramide 18:1;O2/24:0 ratio of patients with and without cirrhosis was comparable ($p > 0.05$).

Ceramide Levels Stratified for SIRS, Sepsis, and Septic Shock, and Comparison to Healthy Controls

Comparable levels of ceramide species were observed in the 29 patients with SIRS, the 33 patients with sepsis, and the 64 patients with septic shock ($p > 0.05$ for all). The LC/VLC ratio of patients with septic shock was higher compared to patients with SIRS ($p = 0.021$). The ratios of the ceramide species 18:1;O2/26:0, 18:0, 20:0 and 24:1 to ceramide 18:1;O2/24:0 levels were similar between patients with SIRS, sepsis, and septic shock ($p > 0.05$ for all).

Of the 126 patients (excluding those with liver cirrhosis), higher levels of the following ceramide species in the patients compared to the healthy controls were observed: Ceramide 18:1;O2/16:0, 18:0, 20:0, 22:0 and 24:1 (Table 2 and Figure 1A). The ceramide levels of the patients for 18:1;O2/23:0, 24:0 and 26:0 were lower than those of the controls (Table 2 and Figure 1A). The LC/VLC ceramide ratio was higher in the patients than in the controls (Table 2). The ceramide 18:1;O2/16:0, 18:0, 20:0 and 24:1 to ceramide 18:1;O2/24:0 ratios were all significantly induced in patients compared to healthy controls (Table 2).

Ceramide Species of Patients with and without COVID-19

Sepsis patients with SARS-CoV-2 infection (21 patients) had similar levels of all ceramide species to patients with other causes of severe illness ($p > 0.05$ for all). However, the LC/VLC ceramide ratio and the ceramide 18:1;O2/16:0 to ceramide 18:1;O2/24:0 ratios of non-COVID-19 patients were higher (Figure 2).

Compared to the control group, patients with confirmed SARS-CoV-2 (excluding those with liver cirrhosis) had higher levels of the following ceramide species: 18:1;O2/16:0, 18:0, 20:0, 22:0, and 24:1 (Table 3). The ceramide 18:1;O2/26:0 level in patients was lower than in the control group (Table 3). The LC/VLC ceramide ratio was higher in the patients than in the controls (Table 3). The ceramide 18:1;O2/16:0, 18:0, 20:0, and 24:1 to ceramide 18:1;O2/24:0 ratios of non-COVID-19 patients were all higher compared to the controls (Table 3).

The serum levels of the different ceramide species were also analysed in an independent cohort of 96 patients with SARS-CoV-2 infection and 17 patients hospitalised for other conditions (see Table S1 for details of this cohort). Patients with SARS-CoV-2 infection had higher levels of ceramide 18:1;O2/16:0, 18:0, 20:0, and 24:1, and lower levels of ceramide 18:1;O2/23:0, 24:0, and 26:0 compared to the non-infected patients with other less severe diseases (Figure 1B).

Table 2 The Median, and in Round Brackets the Minimum and Maximum Levels of Ceramide Species, Total Ceramide Levels, and the Ceramide Ratios Were Determined in Plasma for the Control Group and the Patient Group, After Excluding Patients with Liver Cirrhosis

Ceramide Species nmol/mL	Healthy Controls (N = 23)	Patients (N = 126)	P-value
16:0	0.41 (0.28–0.66)	0.87 (0.27–6.64)	< 0.001
18:0	0.11 (0.06–0.23)	0.40 (0.04–2.66)	< 0.001
20:0	0.16 (0.09–0.30)	0.29 (0.03–2.04)	< 0.001
22:0	1.40 (0.72–2.02)	1.69 (0.25–9.91)	0.030
23:0	1.37 (0.84–2.21)	0.98 (0.16–6.50)	0.001
24:0	5.59 (2.90–8.44)	3.53 (0.59–20.48)	0.001
24:1	1.94 (1.37–3.45)	4.53 (1.10–17.98)	< 0.001
26:0	0.08 (0.04–0.16)	0.04 (0–0.28)	< 0.001
Total ceramides	11.28 (6.82–17.01)	12.49 (2.71–60.78)	0.073
Ratios			
LC/VLC ceramides	0.06 (0.05–0.13)	0.15 (0.08–0.47)	< 0.001
16:0/24:0	0.08 (0.05–0.17)	0.23 (0.10–1.26)	< 0.001
18:0/24:0	0.02 (0.01–0.04)	0.13 (0.03–0.44)	< 0.001
20:0/24:0	0.03 (0.01–0.05)	0.08 (0.03–0.34)	< 0.001
24:1/24:0	0.41 (0.21–0.65)	1.24 (0.46–2.80)	< 0.001

Abbreviation, LC/VLC: long/very long-chain.

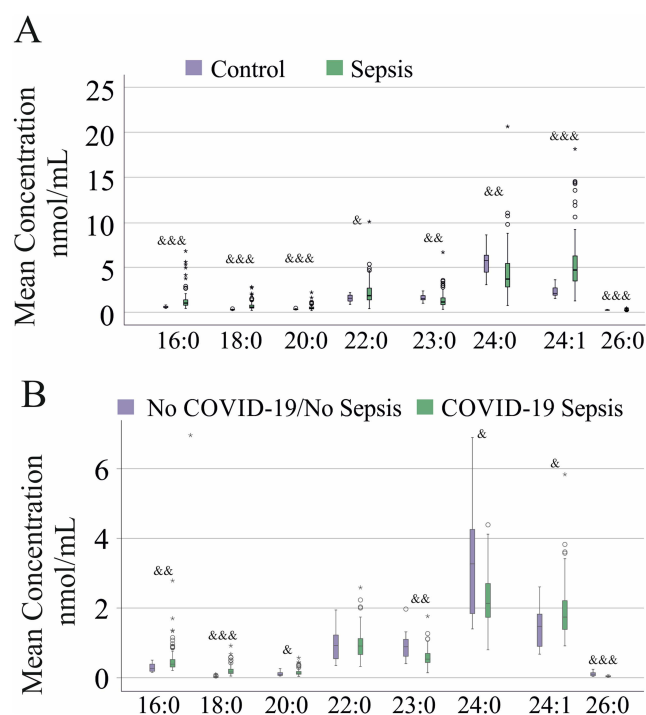


Figure 1 Levels of ceramide species in patients with SIRS/sepsis and controls. **(A)** Ceramides in the plasma of healthy controls and patients with SIRS/sepsis. **(B)** Ceramide levels in the serum of patients without SIRS/sepsis who are not infected by SARS-CoV-2 (non-COVID-19) and SIRS/sepsis patients with COVID-19. The two cohorts enrolled different patients and controls. The small circles and asterisks mark outliers. & $p < 0.05$, && $p < 0.01$, &&& $p < 0.001$.

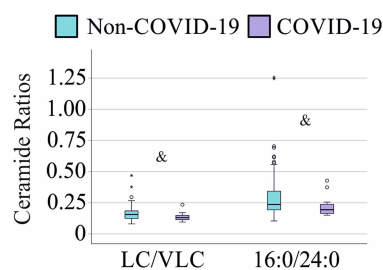


Figure 2 The long/very long-chain (LC/VLC) ceramide ratio and the ceramide 18:1;O2/16:0 to ceramide 18:1;O2/24:0 ratio in patients with SIRS/sepsis, both with and without a diagnosis of SARS-CoV-2 infection. The small circles and asterisks mark outliers. & $p < 0.05$.

The LC/VLC ceramide ratio, the ceramide 18:1;O2/16:0, 18:0, 20:0 and 24:1 to ceramide 18:1;O2/24:0 ratios of COVID-19 patients were higher compared to the non-infected patients with other less severe diseases ($p < 0.001$ for all).

Correlation of Ceramide Species with Markers of Inflammation

In the patients described in Table 1 (excluding those with liver cirrhosis), all but ceramide 18:1/26:0 positively correlated with C-reactive protein (Table 4). Ceramide 18:1;O2/16:0 positively correlated with procalcitonin. None of the ceramides were associated with interleukin-6 (Table 4). A positive correlation between C-reactive protein with the ceramide 18:1;O2/18:0 to 24:0 ratio was observed (Table 4).

Correlation of Ceramide Species with Measures of Liver Disease and Cholesterol

The plasma ceramide species and all of the ratios did not correlate with aminotransferase levels (data not shown). Gamma-glutamyltransferase positively correlated with all ceramide species and bilirubin with ceramide 18:1;O2/16:0 (Table 5). Bilirubin positively correlated with the LC/VLC ratio and the ceramide 18:1;O2/16:0 to ceramide 18:1;O2/24:0 ratio. Albumin levels did not correlate with ceramide species levels but were negatively associated with all of the ratios (Table 5).

Table 3 The Median, and in Round Brackets the Minimum and Maximum Levels of Ceramide Species, the Total Level of Ceramides, and the Ceramide Ratios in Controls and Patients with Confirmed SARS-CoV-2 Infection

Ceramide Species nmol/mL	Healthy Controls (N = 23)	COVID-19 (N = 21)	P-value
16:0	0.41 (0.28–0.66)	0.81 (0.31–2.73)	< 0.001
18:0	0.11 (0.06–0.23)	0.37 (0.19–1.33)	< 0.001
20:0	0.16 (0.09–0.30)	0.29 (0.14–0.67)	< 0.001
22:0	1.40 (0.72–2.02)	1.81 (0.85–4.07)	0.010
23:0	1.37 (0.84–2.21)	1.25 (0.35–2.63)	0.073
24:0	5.59 (2.90–8.44)	4.28 (1.29–8.46)	0.063
24:1	1.94 (1.37–3.45)	4.92 (1.82–7.98)	< 0.001
26:0	0.08 (0.04–0.16)	0.03 (0.00–0.14)	< 0.001
Total ceramide	11.28 (6.82–17.01)	13.43 (5.31–24.79)	0.066
Ratios			
LC/VLC ceramides	0.06 (0.05–0.13)	0.13 (0.10–0.23)	< 0.001
16:0/24:0	0.08 (0.05–0.17)	0.20 (0.15–0.43)	< 0.001
18:0/24:0	0.02 (0.01–0.04)	0.10 (0.05–0.21)	< 0.001
20:0/24:0	0.03 (0.01–0.05)	0.08 (0.05–0.14)	< 0.001
24:1/24:0	0.41 (0.21–0.65)	1.18 (0.69–1.88)	< 0.001

Abbreviation: LC/VLC, long/very long-chain.

Table 4 Spearman Correlation Coefficients Were Calculated for the Correlations Between C-Reactive Protein, Procalcitonin, Interleukin-6, Ceramide Species, Total Ceramide Levels and the Ceramide Ratios in 126 Patients. Patients with Liver Cirrhosis Were Excluded

Ceramide Species nmol/mL	C-Reactive Protein (N = 126)	Procalcitonin (N = 122)	Interleukin-6 (N = 122)
16:0	0.247**	0.188*	0.023
18:0	0.382***	−0.005	−0.062
20:0	0.339***	0.015	−0.053
22:0	0.271**	0.030	−0.081
23:0	0.183*	0.105	−0.150
24:0	0.236**	0.024	−0.123
24:1	0.294**	0.088	−0.020
26:0	0.104	0.159	−0.107
Total ceramide	0.283**	0.079	−0.072
Ratios			
LC/VLC ceramides	0.149	0.068	0.103
16:0/24:0	0.055	0.167	0.153
18:0/24:0	0.226*	−0.020	0.041
20:0/24:0	0.156	−0.069	−0.026
24:1/24:0	0.065	0.054	0.065

Note: *p < 0.05, **p < 0.01, ***p < 0.001.

Abbreviation: LC/VLC, long/very long-chain.

Cholesteryl ester positively correlated with all ceramide species except ceramide 18:1;O2/16:0, and negatively with all but the the ceramide 18:1;O2/18:0 to ceramide ceramide 18:1;O2/24:0 ratio. Free cholesterol positively correlated with all ceramide species (Table 5). It should be noted that positive correlations between several ceramide species and cholesteryl ester and free cholesterol levels were also observed in the control group (Table S3).

Table 5 Spearman Correlation Coefficients Were Calculated for the Correlations Between Markers of Liver Disease, as Well as Cholesterol, with Ceramide Species, Total Ceramide Levels, and the Ceramide Species Ratios

Ceramide Species	Gamma-Glutamyl Transferase (N = 100)	Bilirubin (N = 119)	Albumin (N = 116)	Cholesteryl Ester (N = 126)	Free Cholesterol (N = 126)
16:0	0.417***	0.201*	-0.126	0.157	0.769***
18:0	0.352***	0.039	-0.091	0.277**	0.593***
20:0	0.383***	0.008	-0.040	0.305**	0.641***
22:0	0.316**	-0.006	-0.023	0.327***	0.730***
23:0	0.327**	0.028	0.175	0.391***	0.744***
24:0	0.279**	-0.059	0.114	0.464***	0.730***
24:1	0.338**	0.083	-0.089	0.242***	0.759***
26:0	0.339**	0.036	0.094	0.398***	0.651***
Total ceramide	0.345***	0.034	-0.002	0.343***	0.799***
Ratios					
LC/VLC ceramides	0.178	0.263**	-0.326***	-0.205*	0.095
16:0/24:0	0.118	0.331***	-0.389***	-0.357***	0.065
18:0/24:0	0.060	0.089	-0.282**	-0.162	0.002
20:0/24:0	0.104	0.021	-0.224*	-0.177*	-0.059
24:1/24:0	-0.027	0.170	-0.349***	-0.381***	-0.104

Note: Patients with liver cirrhosis were excluded. *p < 0.05, **p < 0.01, ***p < 0.001.

Abbreviation: LC/VLC, long/very long-chain.

Correlation of Ceramide Species with Age, Body Mass Index and Sex Distribution

In the patients, the plasma ceramide species and the ceramide ratios did not correlate with body mass index ($p > 0.05$ for all). The ceramide species ceramide 18:1;O2/22:0, 24:0, and 24:1 negatively correlated with age (Table S4).

Among the control group, ceramide 18:1;O2/16:0 and 18:0, as well as the LC/VLC ratio, the ceramide 18:1;O2/16:0 to ceramide 18:1;O2/24:0 and the ceramide 18:1;O2/18:0 to ceramide 18:1;O2/24:0 positively correlated with age, while ceramide 18:1;O2/26:0 negatively correlated with age (Table S3).

In the sepsis patients, the ceramide species levels and the total ceramide level were comparable between the 88 men and 38 women ($p < 0.05$ for all). The ceramide 18:1;O2/16:0 to ceramide 18:1;O2/24:0 ($p = 0.012$) and the ceramide 18:1;O2/24:1 to ceramide 18:1;O2/24:0 ($p = 0.043$) of females were increased.

Ceramide Species and Survival

Twenty-five of our patients did not survive. Levels of ceramide species and all the ceramide ratios were not associated with survival (Figure 3 and data not shown). Thirteen patients with liver cirrhosis died, and no associations with ceramides were detected.

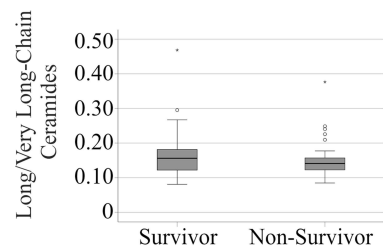


Figure 3 Long/very long-chain ceramide ratio of the 25 patients with SIRS/sepsis who did not survive and patients who survived. The small circles and asterisks mark outliers.

Discussion

Sepsis, irrespective of cause, is characterised by a redistribution of ceramide species. Across sepsis cohorts, five ceramide species increased and three decreased, yielding an overall shift towards long-chain species and a higher LC/VLC ratio. A higher LC/VLC ratio has been linked to adverse outcomes in critical illness,^{46,47} aligning with our observations while underscoring the need for mechanistic studies.

Our results confirmed prior findings in a separate cohort:¹⁴ increases in ceramide 18:1;O2/16:0, 18:0, 20:0 and 24:1, and decreases of ceramides 18:1;O2/23:0 and 24:0. We additionally observed a reduction in ceramide 18:1;O2/26:0, a species not measured previously.¹⁴ The consistent pattern supports a sepsis-associated shift toward long-chain ceramides and a higher LC/VLC ratio, with translational implications that merit targeted mechanistic and interventional studies.

The different ceramide species are produced by six different ceramide synthases: ceramide synthase 1 synthesizes C18 ceramides; ceramide synthase 2 synthesizes C22–26 ceramides; ceramide synthase 3 synthesizes C18, C24 and C26 ceramides; ceramide synthase 4 synthesizes C18–20 ceramides; and ceramide synthases 5 and 6 synthesize C14–16 ceramides.^{46,48,49} It is currently unknown whether the opposite changes of LC and most of the VLC ceramide species in sepsis are related to the altered activity of these enzymes.

We observed a reciprocal pattern: ceramide 18:1;O2/24:0 decreased, whereas ceramide 18:1;O2/24:1 increased in sepsis plasma. The basis for this divergence is unclear; to our knowledge, no pathway has been shown to selectively favor the production or degradation of one of these species over the other.

Patients with coronary artery disease who died have higher serum levels of ceramide 18:1;O2/16:0, 18:0 and 20:0 and reduced ceramide 18:1;O2/24:0 compared to controls who survived.⁵⁰ In non-survivors, the relative differences in these ceramide species versus survivors were modest (8–15%) and notably smaller than the sepsis-associated shifts. In coronary artery disease, the ratios ceramide 18:1;O2/16:0 to 18:1;O2/24:0 and ceramide 18:1;O2/20:0 to 18:1;O2/24:0 predict mortality,^{44,50} however, in our SIRS/sepsis cohort these ratios were not linked to mortality.

In a model of endotoxin-induced septic shock, mice with a knock-out of the ceramidase 2 gene, which renders them unable to generate VLC ceramides, exhibited higher levels of serum tumor necrosis factor. The mortality rate of the mutant mice was much higher than that of the wild-type controls.⁵¹ Overexpression of ceramide synthase 6 was shown to augment the inflammatory response of macrophages, which was reduced upon ceramide synthase 6 knockdown.⁵² These experimental studies provide evidence of the protective function of VLC ceramides and the harmful role of LC ceramides in sepsis.^{51,52} Consequently, the approach of lowering ceramides, regardless of their acyl chain length, appears to be an ineffective strategy for treating sepsis. Moreover, it has been shown that increases in ceramide levels of mice injected with lipopolysaccharide were retained in ASM null mice, showing that this pathway contributes little to higher serum ceramide levels.¹¹ Induction of serine-palmitoyl transferase in the liver of these mice indicates that elevation of ceramides is partly attributed to hepatic de novo synthesis.¹¹

Pharmacological inhibition of ASM impedes SARS-CoV-2 epithelial cell entry in vitro.⁵³ Observational clinical data suggest that antidepressants with high ASM-inhibitory activity (FIASMs), such as fluoxetine, are associated with lower COVID-19 mortality,^{24,25} although in-vivo mechanisms remain incompletely defined. In septic mice, fluoxetine increased IL-10 and improved survival.⁵⁴ To our knowledge, the impact of fluoxetine on circulating ceramide species in patients with COVID-19 has not been characterised. In our cohorts, plasma ceramide species were not associated with mortality. Taken together, these findings argue that any protective effect of fluoxetine in COVID-19 is unlikely to be mediated primarily by global reductions in circulating ceramides, and may instead reflect ASM-dependent effects on viral entry and/or immunomodulation.^{24,25}

The current study showed that ceramide species positively correlated with C-reactive protein, gamma-glutamyltransferase, and cholesterol. This was similar for LC and VLC species, showing that these associations do not contribute to the differential change of LC and most VLC ceramides in sepsis. Moreover, the positive correlation of ceramide species with cholesteryl ester and free cholesterol levels was also significant in the controls, excluding a specific effect of these associations in sepsis.

Several methodological differences may account for discrepancies with prior reports. Abusukhun et al observed increases in C16, C18, C20, C22, C24, and C24:1 ceramides in severe disease versus controls, but the analysis included only 23 severe cases

and five controls, limiting generalizability.¹³ Khodadoust et al reported a 33-fold rise in Cer (d18:1/22:0) in SARS-CoV-2 infection, rising to 50-fold in patients with respiratory distress compared with 18 uninfected controls; however, limited clinical characterization of participants in that study precludes direct comparison with our cohort.¹⁸

Prior work reported higher plasma C16:0, C18:0, C22:0, and C24:1 in critically ill versus moderately ill COVID-19 patients,¹⁹ consistent with our higher levels in severe SARS-CoV-2 compared with non-COVID-19 patients. In contrast, C24:0 was unchanged¹⁹ but declined in our cohorts; C23:0 and C26:0—reduced in our sepsis patients—were not analysed in that study.¹⁹ Overall, studies converge on increased LC ceramides in sepsis, while regulation of VLC species remains inconsistent. Associations with sex, age and BMI were weak or absent, arguing against meaningful confounding.

Consistent with prior work,¹⁷ liver cirrhosis is characterized by broad reductions across ceramide species—including ceramide 18:1;O2/16:0, 18:0, 20:0, 22:0, 23:0, 24:0, 24:1 and 26:0—indicating depletion of both LC and VLC pools. Because cirrhosis imposes a substantial baseline shift in the ceramide milieu, future sepsis lipidomics should pre-specify stratification by cirrhosis and consider sensitivity analyses excluding these patients when interrogating disease-associated lipid changes. The ceramide profile of a large group of patients with both sepsis and liver cirrhosis should be evaluated to see if ceramide species levels could be used as a diagnostic and prognostic tool.

Our study benefits from a comparatively large cohort and a single-centre design that supports procedural consistency, yet generalizability may be limited; multicentre validation will be important. Moreover, we could not adjust for comorbidities known to influence circulating ceramides—type 2 diabetes, cardiovascular disease, and chronic liver disease.^{15,47,55} Common drugs such as statins were found to lower ceramide levels of patients⁵⁶ and their use was not documented. Blood samples were obtained from patients in a non-fasted state, with only one sample collected from each patient at an early stage of the disease. The non-sepsis control cohorts were rather small, so selection bias may have occurred.

Further studies involving detailed phenotyping and pre-specified adjustment or stratification for all the above confounders are warranted, but would require very large patient cohorts.

Prospective mechanistic studies and trials are warranted to test whether pharmacological inhibition of ceramide synthases improves outcomes. Although specific ceramide synthase inhibitors are available,⁵⁷ these drugs have not yet been approved for use in patients. Furthermore, the systemic administration of P053 to inhibit ceramide synthase 1 lowered the levels of ceramide C18:0 and C18:1, while increasing the levels of ceramide 24:0 and 24:1. Over a period of six months, this treatment exacerbated age-related muscular dysfunction.⁵⁸ This experimental study showed that inhibiting specific ceramide synthases can profoundly alter the ceramide profile and may also cause severe side effects. Further studies are needed to determine whether drugs such as P053 provide protection against sepsis and whether adverse events also occur during the shorter treatment courses usually experienced by patients with sepsis.^{58,59}

Conclusion

Across patients with SIRS/sepsis, ceramide profiles were broadly similar irrespective of SARS-CoV-2 infection. In this observational cohort, patterns consistent with increased long-chain ceramides implicate ceramide synthases 1, 5, and 6 as potential therapeutic targets for severe sepsis. However, no data is provided on the efficiency of such interventions.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author, [C.B.], upon reasonable request.

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Author Contributions

Conceptualization: Christa Buechler, Vlad Pavel, Patricia Mester. Data curation: Gerhard Liebisch, Christa Buechler. Formal analysis: Marcus Höring, Sabrina Krautbauer, Gerhard Liebisch, Christa Buechler. Resources: Vlad Pavel, Patricia Mester, Stephan Schmid, Martina Müller. Writing – original draft: Christa Buechler. Writing – review and

editing: Vlad Pavel, Patricia Mester, Marcus Höring, Sabrina Krautbauer, Gerhard Liebisch, Stephan Schmid, Martina Müller, Christa Buechler. All authors gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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