The Journal of Infectious Diseases

MAJOR ARTICLE

Serum Levels of Proinflammatory Lipid Mediators and Specialized Proresolving Molecules Are Increased in Patients With Severe Acute Respiratory Syndrome Coronavirus 2 and Correlate With Markers of the Adaptive Immune Response

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Background. Specialized proresolution molecules (SPMs) halt the transition to chronic pathogenic inflammation. We aimed to quantify serum levels of pro- and anti-inflammatory bioactive lipids in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patients, and to identify potential relationships with innate responses and clinical outcome.

Methods. Serum from 50 hospital admitted inpatients (22 female, 28 male) with confirmed symptomatic SARS-CoV-2 infection and 94 age- and sex-matched controls collected prior to the pandemic (SARS-CoV-2 negative), were processed for quantification of bioactive lipids and anti-nucleocapsid and anti-spike quantitative binding assays.

Results. SARS-CoV-2 serum had significantly higher concentrations of omega-6–derived proinflammatory lipids and omega-6– and omega-3–derived SPMs, compared to the age- and sex-matched SARS-CoV-2–negative group, which were not markedly altered by age or sex. There were significant positive correlations between SPMs, proinflammatory bioactive lipids, and anti-spike antibody binding. Levels of some SPMs were significantly higher in patients with an anti-spike antibody value >0.5. Levels of linoleic acid and 5,6-dihydroxy-8Z,11Z,14Z-eicosatrienoic acid were significantly lower in SARS-CoV-2 patients who died.

Conclusions. SARS-CoV-2 infection was associated with increased levels of SPMs and other pro- and anti-inflammatory bioactive lipids, supporting the future investigation of the underlying enzymatic pathways, which may inform the development of novel treatments.

Keywords. SARS-CoV-2; specialized proresolving molecules; immune response; bioactive lipids; anti-nucleocapsid.

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is characterized by fever and cough, with more severe cases developing acute respiratory distress, acute lung injury, pneumonia, and mortality [1–3]. The higher rates of severe SARS-CoV-2 illness and death are associated with increasing age [4, 5]. SARS-CoV-2 infection is associated with changes in adaptive and innate immunity, including elevated levels of circulating neutrophils and in severe cases the presence of peripherally derived macrophages in the lungs [6, 7], reduced numbers of circulating T cells [8], and robust cytokine responses, which continues after clearance of the virus [9]. SARS-CoV-2 infection is also associated with elevated levels of prostaglandins (PGs) [10, 11], although these levels decrease with severe disease [11], and proinflammatory cytokines, including interleukin (IL) 6, IL-1β, and tumor necrosis factor–α [12, 13]. Importantly, levels of the anti-inflammatory cytokines IL-4 and IL-10 are also elevated following infection [12].

Prostaglandins and leukotrienes have essential roles in initiating acute inflammatory responses and the generation of proinflammatory cytokines, which sustain chronic inflammatory responses. In concert with the cyclooxygenase (COX) pathways, the lipoxygenase (LOX) pathways produce proinflammatory hydroxyeicosatetraenoic acids (HETEs)

Received 21 September 2021; editorial decision 22 December 2021; accepted 2 January 2022; published online 3 January 2022.

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The Journal of Infectious Diseases® 2022;225:2142–54

https://doi.org/10.1093/infdis/jiab632

from arachidonic acid (AA) and hydroxyoctadecadienoic acids (HODEs) from linoleic acid (LA). The active curtailing of inflammatory signalling is essential to restore tissue homeostasis and prevent chronic inflammatory events leading to pathology [14]. Following the initial acute inflammatory phase, specialized proresolving molecules (SPMs), derived from key polyunsaturated fatty acids (PUFAs), are generated and orchestrate the resolution of inflammation by promoting macrophage-mediated clearance of cellular debris and counteracting the effects of proinflammatory cytokines [14]. The SPMs are derived from omega-6 (LA, AA) or omega-3 (eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA]) substrates via the COX, LOX, and cytochrome P450 (CYP) pathways [14] (Supplementary Figure 1). Knowledge of the impact of SARS-CoV-2 infection on the pathways that drive the resolution of inflammation will provide crucial new mechanistic insight and potential novel avenues for treatment [15–17].

The most well-characterized SPMs are the resolvins (Rvs), protectins, and maresins (MaRs), which halt the transition from acute to chronic inflammation preventing pathogenesis [18]. 17(S)-hydroxy docosahexaenoic acid (17-HDHA), a substrate for the generation of the D series resolvins [14], enhances the adaptive immune response in a preclinical model of influenza [19]; both 17-HDHA and RvD1 enhance B-cell production and promote B-cell differentiation towards an antibody-secreting phenotype [20]. The epoxyeicosatrienoic acids (EETs), derived from AA via the CYP pathway (Supplementary Figure 1), also mediate resolution of inflammation [21, 22], down-regulating inflammatory transcription factors such as NF-kB [23], curtailing the induction of COX2 and production of cytokines [24].

Current knowledge of the consequences of SARS-CoV-2 infection on endogenous levels of SPMs and EETs is in its infancy [11, 25–27]. Building a comprehensive picture of the impact of SARS-CoV-2 infection upon circulating levels of bioactive lipids will aid understanding of the therapeutic potential of the resolution pathways for SARS-CoV-2 infection [15, 16, 28]. Our aims were to (1) compare serum levels of a range of SPMs and proinflammatory bioactive lipids between patients admitted to hospital with SARS-CoV-2 infection and an age-matched control group; (2) determine the potential relationship between levels of these bioactive lipids and levels of anti-nucleocapsid and anti-spike antibody binding, markers of the production of an adaptive immune response [29]; and (3) investigate outcomes following infection.

MATERIALS AND METHODS

Sample Collection and Preparation

Serum samples obtained from 50 inpatients admitted to Nottingham University Hospitals National Health Service (NHS) Trust Queen's Medical Centre with symptomatic reverse-transcription polymerase chain reaction–confirmed SARS-CoV-2 infection were collected as diagnostic specimens for clinical chemistry testing. Excess serum was provided anonymously for research purposes, and was the only biofluid available during the height of the pandemic. Review by the University of Nottingham's School of Life Sciences Ethical Review Committee deemed the study to not require full ethical review. Approval for use of anonymized clinical data was provided by the NHS Health Research Authority (HRA) and Health and Care Research Wales (reference number 20/HRA/4843). Samples were determined not to be relevant materials in line with the Human Tissue Authority. Risk assessments were approved by the United Kingdom Health and Safety Executive (reference number CBA1.470.20.1). Serum samples were initially stored at 4°C for 24 hours and then inactivated with the World Health Organization–approved protocol (4-hour room temperature incubation with 1% Triton X-100 in phosphate-buffered saline) before analysis. Detailed methods on the assessment of the potential effect of the viral deactivation protocol on serum lipid levels are provided in the Supplementary Information. Viral genomic sequencing of samples from a subset of these patients was performed as part of the COVID-19 Genomics United Kingdom (COG-UK) consortium [30].

Baseline serum samples from the Internet-Based Exercise Programme Aimed at Treating Knee Osteoarthritis (iBEAT-OA) cohort study (n = 94) were used as age- and sex-matched SARS-CoV-2–negative controls [31]. As these samples were collected prepandemic, a lack of SARS-CoV-2 infection was not confirmed. The iBEAT-OA cohort had a confirmed diagnosis of osteoarthritis, these samples were used as a control group as they were age- and sex-matched and also had a range of comorbidities (Supplementary Table 1). Ethical approval was obtained from the Research Ethics Committee (reference number 18/EM/0154) and the HRA (protocol number 18021).

Lipidomic Analysis

Serum bioactive lipids were extracted and measured using our published liquid chromatography–tandem mass spectrometry quantification method for the major classes of pro- and anti-inflammatory lipid molecules, which has been updated to include SPMs and their precursor molecules [32]. Forty-four bioactive lipids were quantified; detailed methods are shown in the Supplementary Information and Supplementary Table 2.

Anti-Nucleocapsid and Anti-Spike Binding Assays

Anti-nucleocapsid and anti-spike quantitative binding assays were performed on serum samples using enzyme-linked immunosorbent assay (ELISA) following the protocol described by Tighe et al [29]. Levels of C-reactive protein (CRP) were measured in clinical diagnostic tests (mean, 172.9 [range, 11–489 mg/L]). The median time between sample collection for CRP measurement and collection of serum for the measurement of bioactive lipids, anti-nucleocapsid, and anti-spike antibody binding was 5 days.

Data Analysis

GraphPad Prism (version 8.2.1) was used. In some cases, patients were stratified into 3 age groups (≤60, 61–74, and ≥75 years). Groups were assessed for normal distribution using D'Agostino–Pearson test and evaluated for significant changes between groups using Kruskal–Wallis test with multiple comparisons corrected for using Dunn test. Multivariate analysis using Metaboanalyst 4.0 (https://www.metaboanalyst.ca/) [33] including principal component analysis was performed. Partial least squares discrimination analysis (PLS-DA) was used to identify the lipid mediator clusters. A cross-validation analysis was used to validate the PLS-DA model based on accuracy, R^2 and Q^2 scores, and a permutation test. Variable importance in projection (VIP) scores >1 was recognized as playing a key role in cluster differentiation (Supplementary Figure 4 and Supplementary Table 3).

RESULTS

Characteristics of SARS-CoV-2 Infection Cohort and Clinical Features

Serum samples collected from hospital inpatients (22 female, 28 male) with confirmed diagnosis of SARS-CoV-2 (SARS-CoV-2) were studied (Supplementary Table 1). Thirty patients recovered and were discharged, 20 died, and 25 spent time in the intensive care unit during hospitalization (Supplementary Table 1).

Distinct Bioactive Lipid Profile of SARS-CoV-2 Serum

SARS-CoV-2 serum had high concentrations of omega-6–derived proinflammatory lipids and omega-6– and omega-3–derived anti-inflammatory SPM lipids (Table 1), which were significantly increased compared to age- and sex-matched control serum samples. In control sera, comparable to the healthy population [34], many of the anti-inflammatory SPM lipids were not detectable or were present at very low levels. PLS-DA analysis of all lipids quantified identified contributors to the separation between the SARS-CoV-2 and control serum (Figure 1A). Twenty-two lipids from 7 classes of lipids had a VIP score >1 and statistically underpinned the separation between the 2 clusters (Figure 1B). Although serum concentrations of omega-3 (Figure 1C) and omega-6 (Figure 1E) were similar between the SARS-CoV-2 and control sera, concentrations of the downstream bioactive lipids were markedly increased in SARS-CoV-2 serum. There were substantially higher levels of the anti-inflammatory SPMs (17-HDHA, RvD4, LXA4, LXA5) (Figure 1D), PGD2 (Figure 1F), HETEs (Figure 1G), 11,12-EET (Figure 1H), and endocannabinoids (2-arachidonoylglycerol [2-AG], N-arachidonoylethanolamine [AEA]) (Figure 1J) in SARS-CoV-2 serum, compared to control serum. Our findings support a profound mobilization of proresolving and proinflammatory mediators following SARS-CoV-2 infection.

PLS-DA analysis revealed a clear separation between the SARS-CoV-2 and control sera for the 3 age groups (Supplementary Figure 2*A*–*C*). Overall, the majority of the

Table 1. Concentrations of Proinflammatory and Anti-inflammatory Bioactive Lipids Quantified in Patients With Severe Acute Respiratory Syndrome Coronavirus 2 (n = 50) and Age- and Sex-Matched Controls (n = 94)

Bioactive Lipids	Mean Concentration, nM ± SD		*P* Value
	Controls (n = 94)	SARS-CoV-2 (n = 50)	
Proinflammatory			
AA, μM	47.98 ± 12.75	99.61 ± 60.21	<.0001
LA, μM	307.8 ± 109.7	213.79 ± 108.4	<.001
5-HETE	0.99 ± 0.40	158.33 ± 159.17	<.0001
8-HETE	0.81 ± 0.57	3.52 ± 3.32	<.0001
9-HETE	0.15 ± 0.05	16.27 ± 19.02	<.0001
11-HETE	1.77 ± 1.34	19.84 ± 19.95	<.0001
12-HETE	160.89 ± 127.54	162.41 ± 137.29	.651
15-HETE	7.14 ± 4.79	135.18 ± 125.42	<.0001
16-HETE	0.20 ± 0.05	0.22 ± 0.08	.4442
19-HETE	0.37 ± 0.29	188.65 ± 199.17	<.0001
20-HETE	1.04 ± 0.56	69.80 ± 54.66	<.0001
TXB2	21.66 ± 20.33	63.20 ± 80.40	<.0001
11-dehydro-TXB2	1.10 ± 1.47	0.29 ± 0.33	.0001
PGE2	0.37 ± 0.33	0.56 ± 0.41	.0033
PGD2	0.001[a]	1.12 ± 1.34	<.0001
LTB4	0.52 ± 0.72	5.30 ± 0.67	<.0001
13-oxoODE	6.82 ± 5.45	25.85 ± 32.33	.0001
9-oxoODE	0.85 ± 0.96	7.51 ± 10.28	<.0001
9-HODE	6.14 ± 3.03	225.74 ± 308.14	<.0001
13-HODE	12.44 ± 5.72	263.98 ± 363.04	<.0001
Anti-inflammatory			
DHA, μM	36.96 ± 17.63	39.67 ± 27.01	.7135
EPA, μM	37.26 ± 4.72	26.13 ± 21.59	<.001
17-HDHA	1.88 ± 1.46	88.61 ± 107.3	<.0001
14-HDHA	55.53 ± 54.4	93.11 ± 69.82	.0006
18-HEPE	0.49 ± 0.32	10.27 ± 14.16	<.0001
RvD4	0.001[a]	4.11 ± 3.94	<.0001
Maresin 2	0.03 ± 0.01	0.42 ± 0.48	<.0001
LXA4	0.001[a]	2.29 ± 3.15	<.0001
LXA5	0.001[a]	0.52 ± 0.60	<.0001
5,6-EET	0.24 ± 0.38	0.30 ± 0.29	<.0001
5,6-DHET	0.38 ± 0.21	2.35 ± 1.73	<.0001
5,6-Ratio	0.60 ± 0.72	0.21 ± 0.24	<.0001
8,9-EET	0.21 ± 0.21	0.74 ± 0.88	<.0001
8,9-DHET	0.22 ± 0.07	0.47 ± 0.32	<.0001
8,9-Ratio	0.97 ± 0.86	2.63 ± 4.09	.0098
11,12-EET	0.56 ± 0.77	160.95 ± 122.47	<.0001
11,12-DHET	0.53 ± 0.16	0.60 ± 0.32	.315
11,12-Ratio	1.09 ± 1.31	363.01 ± 407.70	<.0001
14,15-EET	0.27 ± 0.35	0.43 ± 0.44	<.0001
14,15-DHET	0.43 ± 0.14	0.52 ± 0.27	.0672
14,15-Ratio	0.68 ± 0.74	0.90 ± 0.73	.0002
AEA	1.80 ± 0.39	1.60 ± 0.77	.009
OEA	7.44 ± 1.92	37.25 ± 15.84	<.0001
PEA	37.53 ± 51.68	29.58 ± 29.82	.079

Data are shown as nM unless otherwise indicated. Statistical analysis by Mann–Whitney test.

Abbreviations: AA, arachidonic acid; AEA, N-arachidonoylethanolamine; DHA, docosahexaenoic acid; DHET, dihydroxyeicosatrienoic acid; EET, epoxyeicosatrienoic acid; EPA, eicosapentaenoic acid; HDHA, hydroxy docosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; LA, linoleic acid; LTB4, Leukotriene B4; LXA4, Lipoxin A4; LXA5, Lipoxin A5; OEA, oleoyl ethanolamide; oxoODE, oxo-octadecadienoic acid; PEA, palmitoyl ethanolamide; PGD2, prostaglandin D2; PGE2, prostaglandin E2; Rv, resolvin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; TXB2, Thromboxane B2.

[a]Where lipids were not detected in samples, an arbitrary value of .001 was used for statistical analysis.

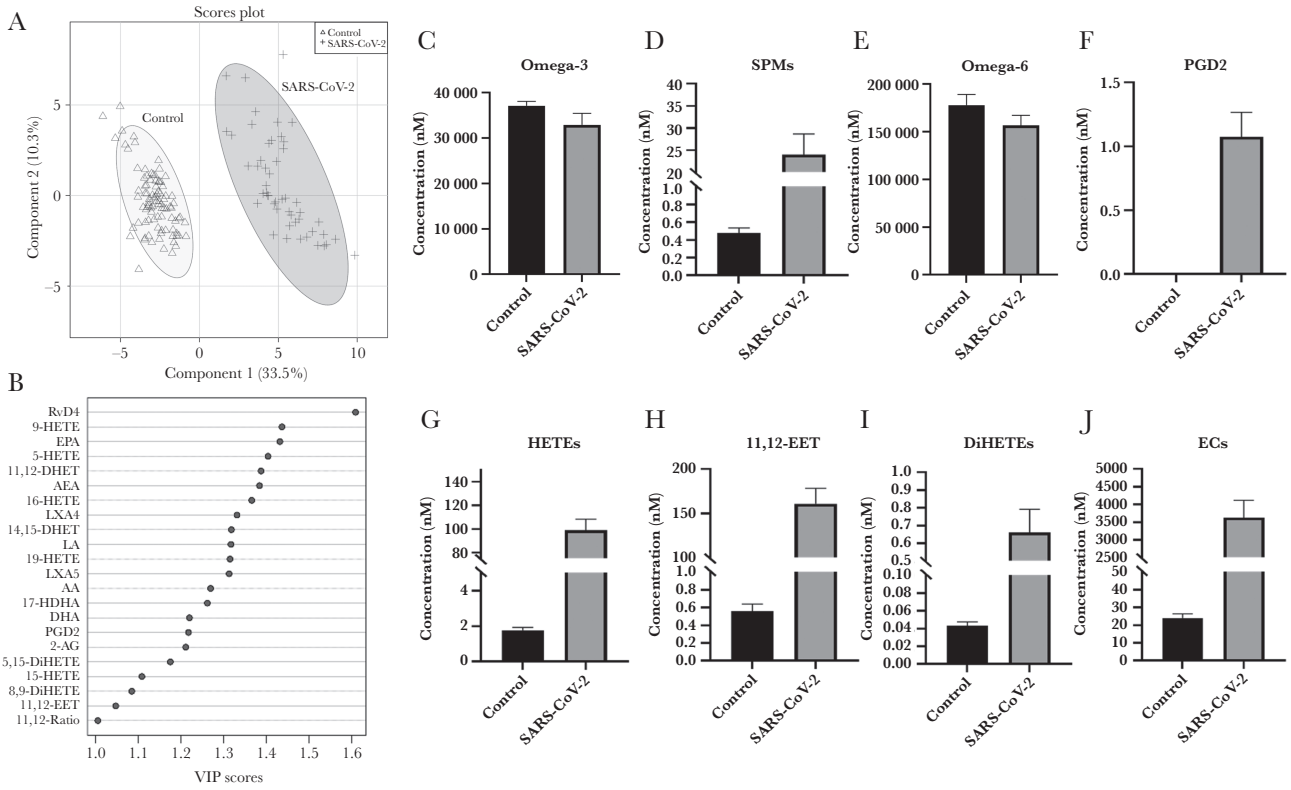

Figure 1. *A*, Partial least square discrimination analysis for the 44 serum lipids quantified in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; n = 50) and age- and sex-matched controls (n = 94) with R^2 = 0.944, Q^2 = 0.932, and accuracy = 1.0. *B*, variable importance in projection (VIP) scores showing important lipid mediators (n = 22, VIP >1.0) involved in differentiation of the 2 groups (control vs SARS-CoV-2). *C–J*, Histograms of the highest-ranked lipid mediators, omega-3 polyunsaturated fatty acids (PUFAs; EPA, DHA), specialized proresolving molecules (17-HDHA, RvD4, LXA4, LXA5), omega-6 PUFAs (AA, LA), PGD2, HETEs (5-HETE, 9-HETE, 16-HETE, 19-HETE, 15-HETE), 11,12-EET, DiHETEs (5, 15-DiHETEs, 8, 9-DiHETEs), and endocannabinoids (2-AG, AEA). Lipid clusters are based on previously reported analysis [34]. Abbreviations: 2-AG, 2-arachidonoylglycerol; AA, arachidonic acid; AEA, N-arachidonoylethanolamine; COVID-19, coronavirus disease 2019; DHA, docosahexaenoic acid; DHET, dihydroxyeicosatrienoic acid; DiHETE, dihydroxyeicosatetraenoic acid; EC, endocannabinoids; EET, epoxyeicosatrienoic acid; EPA, eicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; LA, linoleic acid; LXA4, Lipoxin A4; PGD2, prostaglandin D2; RvD4, resolvin D4; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SPM, specialized proresolving molecules; VIP, variable importance in projection.

lipids contributing to the separation between the SARS-CoV-2 and control sera were the same for the 3 age groups studied (Supplementary Figure 2*D*). This was confirmed by a univariate analysis (Figure 2, Supplementary Tables 4 and 5). Overall, the ability to mount a proresolution response was not markedly altered by age in our study. Analysis of potential sex differences in the levels of the various omega-6–derived proinflammatory lipids and omega-6– and omega-3–derived anti-inflammatory SPM lipids in the SARS-CoV-2 serum vs control was undertaken. However, there were no significant differences between males and females for any of the lipids analyzed (data not shown).

Changes in the flux through enzymatic pathways generating SPMs may provide insight into novel treatments for SARS-CoV-2 infection. Serum levels of DHA and the downstream metabolites, 17-HDHA and 14-HDHA, were correlated in both SARS-CoV-2 and control sera (Figure 3A and 3B). However, levels of EPA and 18-hydroxyeicosapentaenoic acid (18-HEPE) were correlated in SARS-CoV-2 serum but not control serum, suggesting a potential up-regulation of the E series resolving pathway following infection (Figure 3C).

Serum levels of AA were within a healthy range in the control group [35], but were elevated in SARS-CoV-2 serum across all age groups (Supplementary Figure 5*A*). Levels of the anti-inflammatory EETs and the downstream metabolites (dihydroxyeicosatrienoic acid [DHETs]) are presented both individually and as a ratio to reflect the activity of the soluble epoxide hydrolase (sEH) pathway (Table 1). The ratio of 11,12-EET:11,12-DHET was significantly increased in SARS-CoV-2 serum, compared to matched control serum (Supplementary Figure 5*B*). There were some differences in the ratios of 8,9-EET:8,9-DHET and 14,15-EET:14,15-DHET, but these were less consistent (Table 1). In the SARS-CoV-2 serum, levels of AA were correlated with all EETs measured (Figure 4A–D), whereas in the control serum AA was only correlated with 8,9-EET and 11,12-EET (Figure 4B and 4C). These data suggest changes in the flux through the sEH pathway following SARS-CoV-2 infection, worthy of further future investigation.

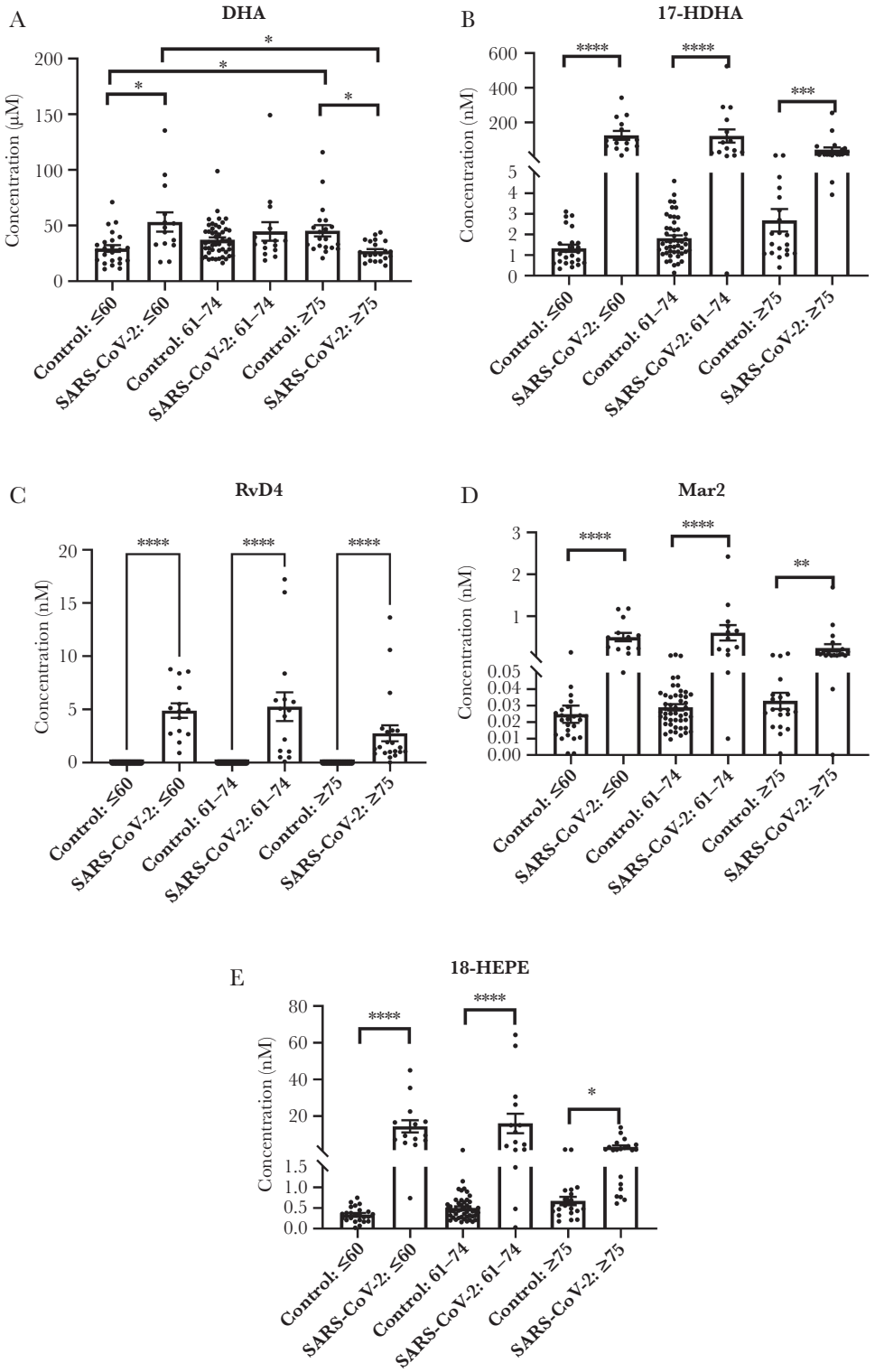

Figure 2. Serum concentrations of docosahexaenoic acid (DHA; *A*), 17-hydroxydocosahexaenoic acid (17-HDHA; *B*), resolvin D4 (RvD4; *C*), maresin 2 (MaR2; *D*), and 18-hydroxyeicosapentaenoic acid (18-HEPE; *E*) in severe acute respiratory syndrome coronavirus 2 (n = 50) and age- and sex-matched control sera (n = 94), stratified by age group. Groups were assessed for normal distribution using D'Agostino–Pearson test. Significance was assessed using Kruskal–Wallis test correcting for multiple comparisons using Dunn test. *$P \leq .05$, **$P \leq .01$, ***$P \leq .001$, ****$P \leq .0001$.

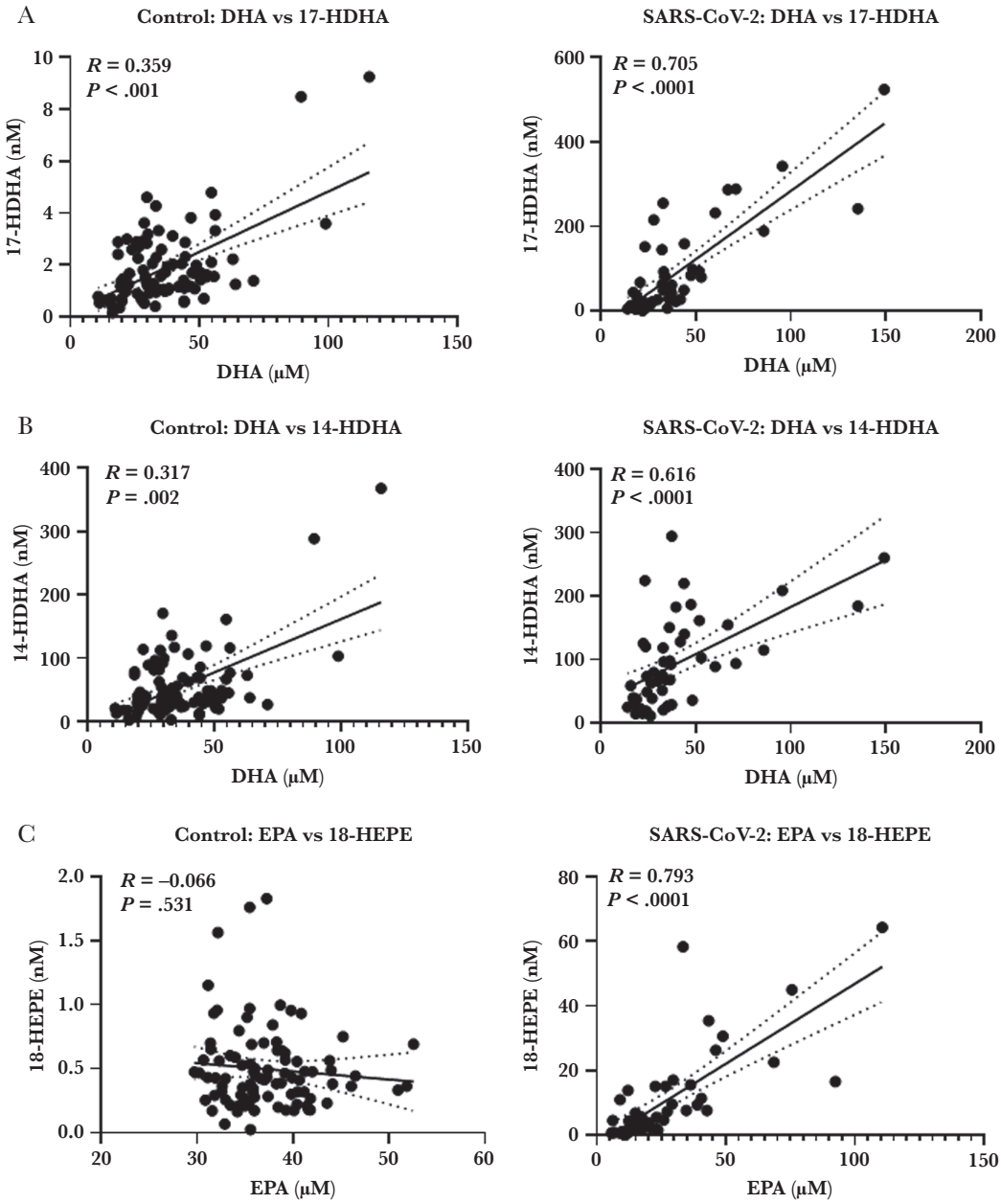

Figure 3. Correlation analysis of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and relevant downstream specialized proresolving molecule pathway metabolites 17-hydroxydocosahexaenoic acid (17-HDHA; *A*), 14-hydroxydocosahexaenoic acid (14-HDHA; *B*), and 18-hydroxyeicosapentaenoic acid (18-HEPE; *C*) in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; n = 50) and age- and sex-matched controls (n = 94). Data analyzed by Spearman ρ.

Associations Between Adaptive Immune Responses to SARS-CoV-2 Infection and Serum Levels of Bioactive Lipids

Anti–SARS-CoV-2 antibody binding to the nucleocapsid protein was interrogated using a reference antigen. Viral sequence data, where available, confirmed that the variants circulating in our cohort were either the Hu-1 strain, or the variant defining the 20B lineage (defined by the spike D614G variant), with no variability in nucleocapsid amino acid sequence [29]. There was a range of levels of anti-nucleocapsid (0.15–3.11 absorbance units [optical density at 450 nm (OD_{450})]) and anti-spike antibody (0.115–3.067 OD_{450}) in the SARS-CoV-2 group.

Based on the ranges, patients were divided into 3 groups of anti-nucleocapsid response (<0.5 OD_{450} [n = 16], 0.5–2.5 OD_{450} [n = 11], and >2.5 OD_{450} [n = 22]) and levels of bioactive lipids between the groups compared. Serum levels of 14-HDHA, 11,12-EET, and 8-, 12-, and 20-HETE were significantly higher in those samples with an anti-nucleocapsid response >2.5 OD_{450} (Supplementary Figure 7). Serum levels of AA, 8-HETE, DHA, EPA, 14-HDHA, and 18-HEPE were positively correlated with anti-nucleocapsid levels (Table 2).

Based on the range of anti-spike antibody binding, patients were separated into 2 groups: low (<0.5 [n = 26]) and high

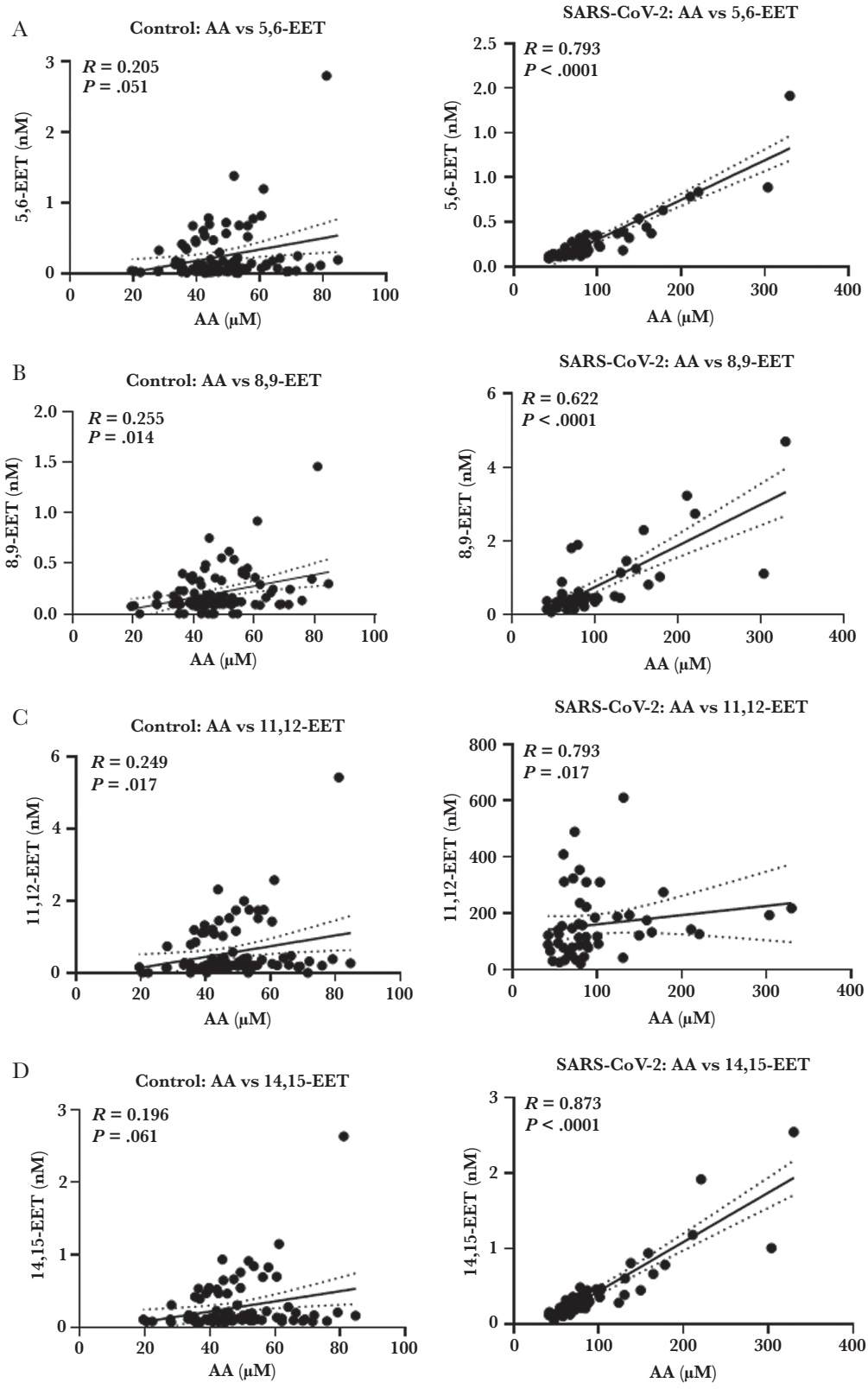

Figure 4. Correlation analysis between arachidonic acid (AA) and downstream cytochrome P450 metabolites 5,6 epoxyeicosatrienoic acid (EET; *A*), 8,9-EET (*B*), 1,12-EET (*C*), and 14,15-EET (*D*) in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and control groups. Data analyzed by Spearman ρ.

Table 2. Correlation Analysis Between Concentrations of Bioactive Lipid With Anti-Nucleocapsid and Anti-Spike Antibody Response in Severe Acute Respiratory Syndrome Coronavirus 2 (n = 50)

	Correlation Analysis of Lipids vs Antibody Binding			
	Anti-Nucleocapsid		Anti-Spike	
Bioactive Lipids	*R* Value	*P* Value	*R* Value	*P* Value
AA	0.326	**.021**	0.352	**.012**
LA	0.249	.081	0.382	**.006**
DHA	0.369	**.009**	0.337	**.017**
EPA	0.419	**.002**	0.540	**<.0001**
17-HDHA	0.269	.059	0.537	**<.0001**
18-HEPE	0.366	**.009**	0.410	**.003**
14-HDHA	0.434	**.002**	0.293	**.039**
RvD4	0.211	.142	0.449	**.001**
Maresin 2	0.282	.055	0.343	**.018**
LXA4	0.051	.723	0.324	**.022**
LXA5	0.222	.133	0.436	**.002**
5-HETE	0.093	.52	0.361	**.010**
8-HETE	0.404	**.004**	0.419	**.002**
9-HETE	0.27	.058	0.469	**.001**
11-HETE	0.279	**.05**	0.464	**.001**
12-HETE	0.231	.106	0.084	.563
15-HETE	0.259	.069	0.407	**.003**
16-HETE	0.245	.097	0.045	.756
19-HETE	0.138	.344	0.314	**.028**
20-HETE	0.234	.101	0.099	.494
TXB2	–0.26	.071	–0.052	.722
PGE2	0.049	.744	0.275	.056
PGD2	0.113	.445	0.283	.052
LTB4	–0.041	.776	0.326	**.021**
5,6-EET	0.357	**.011**	0.271	.057
5,6-DHET	0.024	.867	0.310	**.029**
5,6-Ratio	0.243	.089	–0.018	.904
8,9-EET	0.099	.494	0.185	.198
8,9-DHET	0.063	.663	–0.104	.474
8,9-Ratio	0.064	.664	0.164	.257
11,12-EET	0.239	.093	0.084	.564
11,12-DHET	0.109	.452	–0.041	.779
11,12-Ratio	0.15	.297	0.114	.431
14,15-EET	0.302	**.033**	0.334	**.018**
14,15-DHET	0.023	.874	–0.194	.178
14,15-Ratio	0.339	**.016**	0.443	**.001**
13-oxo-ODE	0.202	.169	0.447	**.002**
9-oxo-ODE	0.243	.154	0.482	**.003**
9-HODE	0.265	.063	0.505	**.0002**
13-HODE	0.268	.059	0.488	**.0003**
AEA	0.017	.907	–0.216	.131
PEA	–0.071	.626	–0.083	.567
OEA	0.063	.666	–0.060	.679

Data analyzed by Spearman ρ. *P* Values <.05 are highlighted in bold.

Abbreviations: AA, arachidonic acid; AEA, N-arachidonoylethanolamine; DHA, docosahexaenoic acid; DHET, dihydroxyeicosatrienoic acid; EET, epoxyeicosatrienoic acid; EPA, eicosapentaenoic acid; HDHA, hydroxy docosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; LA, linoleic acid; LTB4, Leukotriene B4; LXA, Lipoxin (LX); OEA, oleoylethanolamide; oxoODE, oxo-octadecadienoic acid; PEA, N-palmitoylethanolamide (PEA); PGD2, Prostaglandin D2; PGE2, Prostaglandin E2; Rv, resolvin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; TXB2, Thromboxane B2.

(>0.5 [n = 26]), the subset of patients who died had significantly lower levels of anti-spike antibody binding (Supplementary Figure 6). Levels of 18-HEPE, 17-HDHA, RvD4, and 14,15-EET were significantly higher in patients with an anti-spike antibody value >0.5 (Figure 5). There were statistically significant positive correlations between these lipids and anti-spike antibody binding for the entire group of patients (Table 2). Of the lipids measured in the SARS-CoV-2 cohort, 12-, 16-, and 20-HETE;

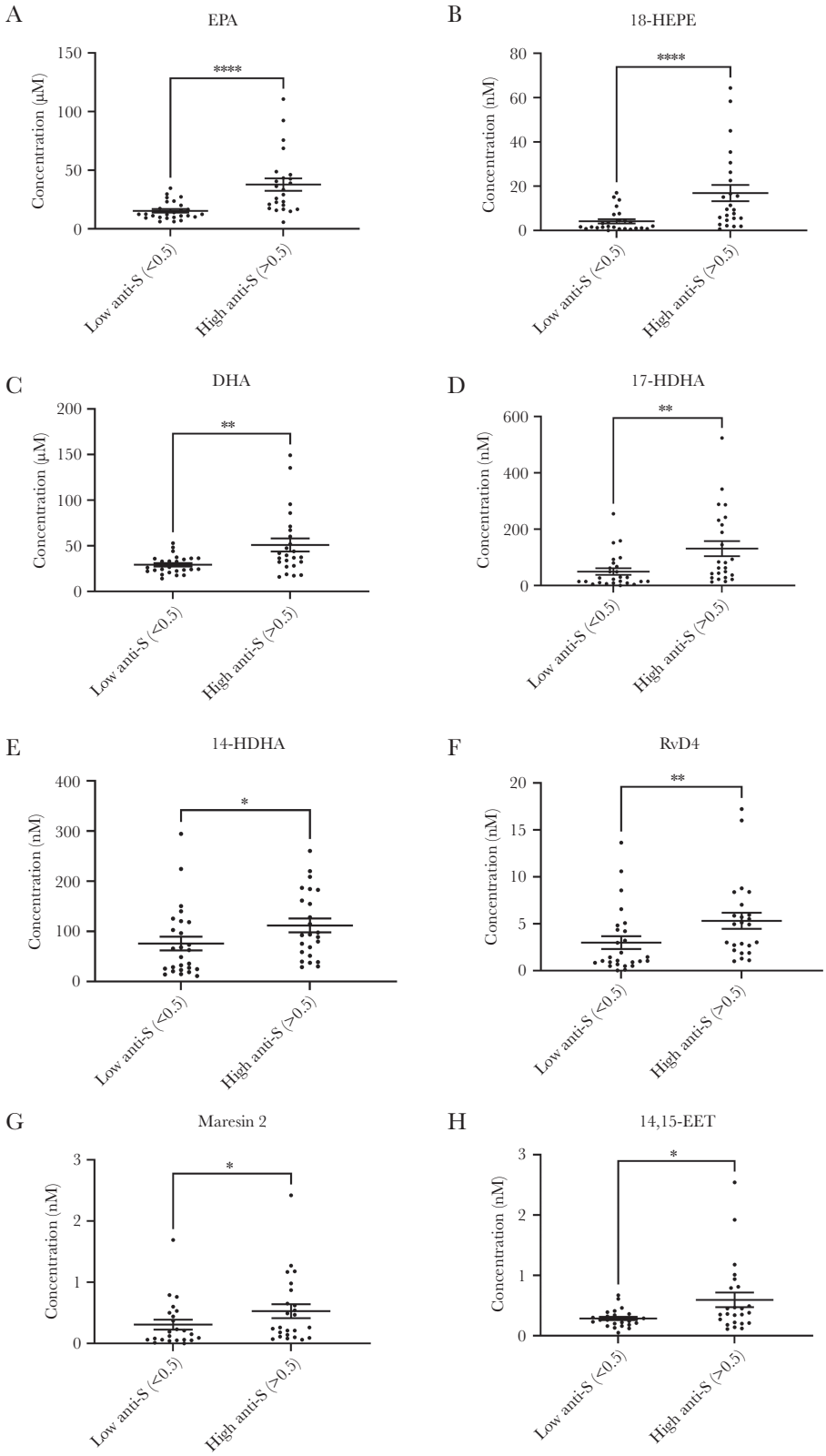

Figure 5. Serum concentration of significantly altered proresolution lipid mediators eicosapentaenoic acid (EPA; *A*), 18-hydroxyeicosapentaenoic acid (18-HEPE; *B*), docosahexaenoic acid (DHA; *C*), 17-hydroxydocosahexaenoic acid (17-HDHA; *D*), 14-hydroxydocosahexaenoic acid (14-HDHA; *E*), resolvin D4 (RvD4; *F*), maresin 2 (*G*), and 14,15-Epoxyeicosa-5,8,11-trienoic Acid (14,15-EET; *H*) based on the anti-spike antibody response (low group <0.5, n = 26; high group >0.5, n = 24) in patients with severe acute respiratory syndrome coronavirus 2. Groups were assessed for normal distribution using D'Agostino–Pearson test. Significance was assessed using Mann–Whitney test. $*P \leq .05$, $**P \leq .01$, $****P \leq .0001$.

Thromboxane B2 (TXB2); PGs; DHETs; endocannabinoids; and 5,6-, 8,9-, and 11,12-EET were not correlated with levels of either anti-nucleocapsid or anti-spike antibody binding values (Table 2).

Analysis of serum levels of bioactive lipids early in the infection and the clinical outcome identified that levels of LA and 5,6-DHET were significantly lower in SARS-CoV-2–infected patients who died, and the ratio of 5,6-EET:5,6-DHET was higher in those patients that died compared to those that survived (Supplementary Table 6).

DISCUSSION

SARS-CoV-2 infection was associated with robust increases in serum levels of both omega-6– and omega-3–derived bioactive lipids, which have well-described pro- and anti-inflammatory roles [36]. Significant increases in serum levels of proinflammatory lipids included PGE2 (1.5-fold), TXB2 (3-fold), Leukotriene B4 (LTB4) (10-fold), 5-HETE (159-fold), and 13-HODE (20-fold) in the SARS-CoV-2 group, compared to the age-matched control group. For the anti-inflammatory bioactive lipids and precursors, there was a 47-fold increase in levels of the SPM precursor 17-HDHA in the SARS-CoV-2 group. Serum levels of the SPMs RvD4 and MaR2 are normally close to, or below, the limits of detection in the healthy population [37], but were present in the SARS-CoV-2 group. Substantial increases in levels of some of the EETs (11,12-EET: 285-fold increase; 8,9-EET: 3.5-fold increase) in the SARS-CoV-2 group suggest a concerted anti-inflammatory response via multiple enzymatic pathways following SARS-CoV-2 infection. Our data support a robust activation of the resolution pathways following SARS-CoV-2 infection, irrespective of patient age, which was not influenced by gender. These data, and an earlier report of elevated levels of RvE3 [11], point to a complex pathophysiological response to SARS-CoV-2 infection, which may be amenable to pharmacological intervention and provide new targets for treatment.

Our findings are consistent with the report of higher levels of plasma and serum SPMs and increased expression of related enzymatic pathways in peripheral blood monocyte subsets in 19 patients infected with SARS-CoV-2 [26]. Increased levels of proinflammatory bioactive lipids and anti-inflammatory SPMs, including RvD4, RvD5, RvD2, RvD1, and PDX, have also been reported in bronchoalveolar lavage from SARS-CoV-2 patients [25]. SPMs are already known to modulate acute lung injury and respiratory distress syndrome, supporting these findings following SARS-CoV-2 infection. Antibodies generated by B cells are critical to antiviral immunity. The D series precursors and resolvins, including 17-HDHA, enhance human B-cell antibody production by promoting differentiation toward an antibody-secreting phenotype [20]. In a preclinical murine model of influenza immunization, 17-HDHA treatment increased antigen-specific antibody responses and protected against live influenza virus infection [19]. These data suggest that a robust generation of 17-HDHA following infection may not only act to counter proinflammatory responses, but also facilitate the response of B cells to mount an antibody response. To date there are no studies of the effects of the SPMs on SARS-CoV-2 infection in patients; however, it has been reported that both RvD1 and RvD2 have beneficial effects on inflammatory responses in SARS-CoV-2–infected macrophages [27].

There was a broad range of anti-nucleocapsid and anti-spike responses in the SARS-CoV-2 group, indicative of adaptive immune response to infection. Consistent with a larger study [29], increased anti-spike responses were associated with improved clinical outcome. SARS-CoV-2 patients with higher anti-spike responses (>0.5) had significantly higher levels of anti-inflammatory/resolution molecules (18-HEPE, 17-HDHA, 14-HDHA, RvD4, MaR2, 14,15-EET), which either directly mediate resolution of inflammation or are metabolites in the resolution pathways, as well as some proinflammatory lipids (LTB4; 5-, 8-, 9-, 11-, and 15-HETE; and 9- and 13-HODE). Levels of PUFA substrates were not substantially altered by SARS-CoV-2 infection or age, so it is unlikely that substrate and therefore diet is a major determining factor in the resolution response to SARS-CoV-2 infection. The strong correlations between PUFAs and their downstream SPM pathway metabolites in the SARS-CoV-2 infection group suggests that these enzymatic pathways are upregulated by this infection, particularly evident for the E series pathway. Our findings support future studies of the relationship between the antibody response to SARS-CoV-2 infection, activation of the resolution pathways, and clinical outcome in a larger cohort of patients.

Current evidence suggests that treatments for SARS-CoV-2 infection, alongside vaccination, will remain a priority in the immediate future. Identification of the critical steps involved in the up-regulation of these anti-inflammatory pathways may be instructive for the development of interventions aimed at dampening the inflammatory response or promoting the clearance of the inflammation arising from SARS-CoV-2 infection. Dexamethasone has been identified as an important treatment to promote recovery from SARS-CoV-2 infection. Dexamethasone increased SPM levels in a small number of SARS-CoV-2–infected patients [26], in healthy volunteers [38], and in allergic airway inflammation [39]. Future investigation of the potential contribution of SPMs to the beneficial effects of dexamethasone in patients with SARS-CoV-2 infection in a larger cohort will improve our understanding of the potential mechanisms of action of this treatment.

There are a number of study limitations. Serum samples were collected from patients hospitalized with SARS-CoV-2 for clinical diagnostic tests during the first wave of the pandemic in the United Kingdom. Due to the clinical pressures within the system at the time, some clinical data, such as body mass index

(BMI) and medication, were not collected. It is known that levels of SPMs are decreased with increased BMI, which could potentially contribute to SARS-CoV-2–related morbidities and mortalities [40]. Although our analysis of SARS-CoV-2 serum (the only biofluid available at the time) may have an impact on the absolute levels of the lipids measured, this was controlled for in our use of SARS-CoV-2–negative serum to mitigate any major impact. Data relating to whether a patient was admitted to intensive care were available for these samples; however, clinical decision making was related to multiple factors beyond the severity of the infection and therefore further analysis of potential impact has not been performed. Serum samples were collected within the first few days of hospital admission and represent a snapshot of the anti-nucleocapsid and anti-spike response and the lipid levels at a point in time. However, levels of antibodies change over time and we do not have matched longitudinal data. The anti-nucleocapsid and anti-spike signal provides an indication of the potency of the adaptive immune response following infection. The viral genome sequencing data available for these patients indicated that the nucleocapsid amino acid sequence was completely conserved between infections. While it is possible that there may be mismatches between the antigen used in our assay and the strain of infecting virus that mean that antibodies are present which are undetected by our assay, at the time of sampling there was minimal genetic diversity in United Kingdom isolates (www.nextstrain.org). Nevertheless to mitigate against this, we used ELISAs against 2 antigens.

In summary, our findings highlight that SARS-CoV-2 infection can lead to very robust activation of the pathways that generate the SPMs and other pro- and anti-inflammatory bioactive lipids. This new knowledge supports the future investigation of these pathways, which may inform the development of novel anti-inflammatory treatments for SARS-CoV-2 infection. Furthermore, these new datasets provide us opportunities to explore further the underlying molecular pathways that regulate the resolution pathways in humans, with the goal of identifying novel approaches for the development of new therapeutics for other infections and chronic inflammatory diseases.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. V. C., D. A. B., D. H. K., and A. W. T. conceived the study, secured the funding, supervised data collection and analysis, and prepared the manuscript. J. T., R. R. J., and C. O. performed lipidomic analysis of samples. A. W. T., P. J. T., and W. L. I. supplied clinical samples, performed measurement of anti-nucleocapsid, and prepared the manuscript. A. M. V. and S. A. G. provided control serum samples. J. T. and R. R. J. performed statistical analysis and prepared the manuscript. E. L. provided clinical insight into data interpretation and prepared the manuscript. All authors reviewed the manuscript and had final responsibility for the decision to submit for publication.

Acknowledgments. The authors thank Professors David Walsh and John Gladman for their advice in preparation of the manuscript. The authors also thank Dr Divyateja Hrushikesh and colleagues from the Queens Medical Centre for providing samples and clinical data.

Disclaimer. The source of funding had no influence over the design or conduct of the study. The views expressed are those of the author(s) and not necessarily those of the National Health Service, the National Institute for Health Research (NIHR), or the UK Department of Health and Social Care.

Financial support. This research was funded by the NIHR Nottingham Biomedical Research Centre.

Potential conflicts of interest. All authors: No reported conflicts of interests.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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