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Acute severe SARS COVID-19 patients produce pro-resolving lipids mediators and eicosanoids

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Abstract. – OBJECTIVE: This study aimed to evaluate the eicosanoid and pro resolutive parameters in SARS COVID-19 patients with the severe acute respiratory syndrome.

PATIENTS AND METHODS: Fourteen male patients with an acute respiratory syndrome caused by SARS COVID-19 and four healthy controls were evaluated by measuring the following parameters in plasma: Polyunsaturated fatty acids: EPA, DHA, ARA, and DPA. Specialized Pro-resolving mediators (SPMs) (including monohydroxy-containing precursors 17-HDHA, 18-HEPE, 14-HDHA) resolvins, maresins, protectins, and lipoxins. The eicosanoids group included prostaglandins, thromboxanes, and leukotrienes.

RESULTS: Plasma from COVID-19 patients presented higher amounts of pro-inflammatory and pro-thrombotic lipid mediators as compared to healthy subjects (65.7 pg/ml *vs.* 10.2 pg/ml), including thromboxane (2142.6 pg/ml *vs.* 10.4 pg/ml), and the ratio between total plasma pro-inflammatory mediators versus total SPM's was 13.2 to 0,4, respectively.

CONCLUSIONS: A clear disbalance favoring the pro-inflammatory axis is described, showing the need to perform future clinical interventions in these patients using SPM's or monohydroxylated lipid mediators derivates from fatty acids.

Key Words:
SARS COVID-19, Specialized pro-resolving mediators, Inflammation, Resolution.

Introduction

Emerging in China in January 2020, coronavirus Sars-cov-2 has provoked the first pandemic of the 21st century: COVID-19 (coronavirus disease 2019) has spread worldwide within few months and still imposes an enormous burden on health systems and the economy. Transmission of the virions mainly occurs via droplet infection. Still, as they remain infective for up to 3 days depending on the environmental conditions, they may also reach their hosts via everyday objects like computer keyboards, door handles, or furniture, finally entering cells of the oral or nasal mucosa or via the conjunctiva of the eye[1].

There is a wide variety in the clinical manifestation of COVID-19, ranging from symptomless infections through intermediate courses of the disease up to life-threatening manifestations with severe pneumonia, multiorgan failure, and death. Both mild and severe forms of COVID-19 may also lead to so-called "Long COVID," a term used for various symptoms caused by the disease that continues for months after the initial infection[2].

Globally, mortality of the disease is about 3.4 %[3], reaching up to 4.3% in Wuhan (China), where COVID-19 has its origins[4]. Comorbidities, mainly Hypertension, and diabetes are directly linked to poor outcomes of the disease[5]. To date, as no effective treatment options against COVID-19 have been developed, only symptomatical approaches are in the management of this disease. Antiviral drugs examined so far have not revealed convincing clinical efficacy yet[6] or still need extensive investigation, such as ivermectin[7]. The majority (80%) of symptomatic patients do not experience life-threatening man-

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ifestations of COVID-19. Still, moderate courses of the disease can quickly become severe, leading to acute respiratory distress syndrome (ARDS) with multiorgan failure and death when no medical treatment occurs. Therefore, patients with moderate symptoms should also receive supportive treatment, including antiviral and/or antiphlogistic drugs, to prevent an aggravation of the disease. The treatment options under investigation include arbidol, chloroquine phosphate, ribavirin, favipiravir, ivermectin, interferon alpha-2b, and treatment with dexamethasone. Treatments with the plasma of convalescents or monoclonal antibodies like etesevimab and bamlanivimab are investigated for their safety and efficacy in Covid-19 patients[6,7]. However, as mentioned above, to date, treatment is restricted to supportive and adjuvant care[8-11].

In many respects, SARS-cov-2 is comparable to the influenza virus, as both are RNA viruses that provoke respiratory symptoms ranging from very mild to highly severe forms that may result in a fatal course of the disease. Severe pathologies are often linked to overshooting reactions of the hosts' immune system, mirrored by the so-called "cytokine-storm". This phenomenon is known for pathogens like the influenza virus or the gram-negative bacterium *Francisella tularens*. It leads to pneumonia or hypercoagulation and it is also observed in COVID-19 disease. Consequently, therapeutically suppressing the inflammatory immune response or the systemical use of active anticoagulants may represent promising approaches in managing COVID-19[8].

Interestingly, it is not only the ARDS mentioned above that is associated with a poor outcome of the disease: in hospitalized COVID-19 patients, myocardial problems and kidney failure are often observed to contribute to a fatal course of the disease[12-14]. However, the basic pathophysiological mechanisms of Sars-cov-2 infections, which are responsible for the damage of the various tissue types, are not entirely understood yet[15]. Coagulation processes seem to play an essential role in this context[16,17]. Still, a systematic description of the underlying coagulatory and fibrinolytic processes and their relationship to the outcome of the disease has not been accomplished yet[18,19]. Therefore, the exact mechanisms by which Sars-cov-2 induces coagulatory and inflammatory responses and the interaction between these pathways during COVID-19 disease are unclear.

Generally, cessation and resolution of inflammatory processes depend on active strategies, which are to a large extent driven by particular lipid mediator (LM) molecules, the so-called specialized pro-resolving mediators (SPM)[20]. They are synthesized by cells of the innate immune system, which utilize the essential fatty acids arachidonic acid (ARA), eicosapentaenoic acid (EPA), n-3-docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) as substrates for enzymatic conversion to form four families of SPMs: lipoxins, resolvins, protectins and maresins[21-23]. All SPMs are involved in actively regulating and enforcing the resolution of inflammatory processes. Due to their action, for example, the amount of pro-inflammatory cytokines and chemokines is reduced at infected sites, and the influx of neutrophils is actively limited. Furthermore, macrophages are stimulated to enhance phagocytosis, killing bacteria and clearance of cell debris[20,22,23]. In animal disease models, an organ-protective action of SPMs has also been demonstrated[22]. Of particular interest in the present context is the observation that SPMs also seem to positively impact the alveolar fluid clearance (AFC) in ARDS, thereby supporting the reconstitution of the physiological function of the lung[24].

When lung tissue is injured, an immune response is triggered, which leads to an increase in the amount of pro-inflammatory molecules at the site of injury, followed by the entrance of immunocompetent cells into the alveolar space. Concomitantly, however, the generation of the pro-resolving SPMs – resolvins, protectins, maresins, and lipoxins – is initiated[25]. The influenza-A-virus demonstrated a direct correlation between its virulence and the profound and continuous induction of the inflammatory response.

Its ability to disseminate into different tissues was not only associated with strong activation of genes encoding for crucial elements of the pro-inflammatory cascade but was also accompanied by a down-regulation of genes responsible for the lipoxin-mediated anti-inflammatory signaling pathways, thereby reducing the pro-resolutive capacity and protective role of the SPM[26]. Also, for Sars-cov-2 patients, a relationship between lipid mediator profile and severity of the disease has been demonstrated recently. Striking differences between the lipid profiles and the abundance of certain LM-derivatives were observed between severe and moderate courses of illness. A relationship between pre-existing comorbidities like

BMI, diabetes, and heart disease and the lipid profile changes has been described. It was speculated whether those risk factors led to a pre-existing imbalance in the LM profiles that might finally contribute to the severity of Covid-19 disease due to the decreased ability to counteract the inflammatory response induced by Sars-cov-19 infection[27].

The present work established comprehensive lipid- and LM profiles of Covid-19-patients compared to healthy persons, and significant alterations were observed in serum and plasma samples. This is of particular interest since coagulation parameters can be determined in plasma samples while not being analyzed in serum. The study aimed at describing the immunological capacity and inflammatory response of COVID-19 patients on the lipidome level compared to healthy individuals by establishing profiles of the LM and their precursor molecules in plasma and sera of the test groups.

Patients and Methods

Blood Samples from COVID-19 Patients

Fourteen male subjects, who had a positive PCR test for SARS COVID-19, and four healthy men without any known acute disease and unsuspicious anamnesis, were included in the study. Between the groups, the BMI was similar. All COVID-19 patients showed a severe pulmonary respiratory syndrome, according to the internationally acknowledged definitions of pneumonia, and were treated in the Department of Respiratory Diseases in the General Hospital in Talca, Chile, following a standardized therapeutic scheme that included low-dose heparin, corticosteroids (4 mg dexamethasone per day), antidepressants and antibiotics. Blood samples were drawn under fasting conditions (8 a.m.) On three consecutive days to get an impression of the temporal changes of the parameters. At sampling, the COVID-19 patients were all in a steady state regarding their clinical status, and none of them received artificial ventilation. Their age ranged between 24 and 72 years, and the healthy subjects were between 36 and 42 years old. Between both groups, the mean BMI did not differ. In Table I, the demographic data are represented.

The trial protocol was designed and conducted following the ethical principles defined in the Declaration of Helsinki, and all procedures were consistent with GCP and the applicable regulatory rules.

Ethical Approval

The investigational trial and center were approved the Comite Etico Cientifica del Servicio de Salud Maule /Chile on 19th June 2020: Clinical Trial registration number: DRKS-ID: DRKS00022337. Date of registration: 29th Jun 2020.

Analytical Procedure

Blood samples were drawn on three consecutive days, with each sample being treated as a mono-replicate. They were separated into plasma and serum, subjected to standard preparation procedures, and stored at -80°C until further analytical processing. All samples were analyzed individually, and the results were used for statistical analysis (see chapter below).

Extraction and Profiling of Lipids and Lipid Mediator by LC-MS/MS

The laboratory analyses were performed at Solutex GC SL.

Extraction of lipid mediators from plasma and serum samples followed a solid phase extraction (SPE) process (see below). For that, the samples were thawed on ice and internally labelled standard solutions of d8-5-HETE, d5-rvd2, d5-LXA4, d4-LTB4, d4-PGE2 (500 pg each, Cay-

Table I. Demographical data of the study population.

	Healthy (n = 4)	COVID-19 (n = 14)
Sex	Male (4)	Male (14)
Age (years)	38 (36-42)	54.1 (24-72) *
BMI (kg/m^2)	31 (26.1-34.6)	32.8 (25.6-44.1)
COVID-19 PCR test	NA	+ (14)
Severity	NA	14
Days in Hospital	NA	23.3 (9-98)
Pneumonia-Respiratory Insufficiency	NA	14

NA = not applicable, mean (range), += positive PCR *$p < 0.05$.

man Chemical Company in 4 ml of methanol (Methanol Optima LC/MS Grade, Fisher Chemical) were mixed with each 1 ml of plasma or serum, thereby allowing for quantification of the detected analytes. Afterward, proteins were removed by precipitation (storage at -80ºc for 30 minutes), followed by a centrifugation step (2000 g, 10 min, 4°C), and the supernatants used for further study analysis were removed. For SPE, established protocols[28,29] were used that include a rapid acidification step (mixing the samples with 9 ml acidic water (HCL); ph = 3.5) before adding them onto the SPE column (100 mg, 10 ml, Biotage). After a neutralization step (4 ml miliq water) and washing of the columns (4 ml n-hexane), elution from the column was done with 9 ml methyl format. SPE extracts were then dried with a gentle nitrogen stream before resuspension in methanol/water (50:50 vol/vol) (meoh/Water Optima LC/MS Grade, Fisher Chemical, both) and injection into the LC-MS/MS system.

Acquisition Parameters of Targeted LC-MS/MS

An LC-MS/MS system with Qtrap 5500 (Sciex) equipped with a Shimadzu LC-20AD HPLC pump. A Kinetex Core-Shell LC-18 column (100 mm × 4.6 mm × 2.6 µm, Phenomenex) was maintained at 50°C, and a binary eluent system of LC-MS/MS grade water (A) (Fisher Chemical) and LC-MS/MS grade methanol (Fisher Chemical) (B), both with 0.01 % (v/v) of acetic acid served as mobile phase. Lms were eluted in a gradient program (with respect to the composition of solvent B) as follows: 0-2 min, 50%; 2-14.5 min, 80%; 14.6-25 min; 98% and a flow rate of 0.5 ml/min.

Negative ionization mode was used to operate the QTRAP 5500 and scheduled Multiple Reaction Monitoring (MRM) coupled with the information-dependent acquisition (IDA). An enhanced Product Ion scan (EPI) was utilized. According to established procedures[29,30], each LM parameter (CE, target retention time (RT), and specific Q1 and Q3 mass) was optimized. For quantification of the analytes, the area under the peaks was calculated.

An MRM with MS/MS matching signature ion fragments for each molecule (at least six diagnostic ions; <0.1 picograms was considered below the limit of detection) was used, applying published criteria[30]. In Figure 5, examples of representative MRM spectra are presented.

Statistical Analysis

For each patient and analyte, arithmetic means, standard error; and minimum and maximum values were calculated displayed. GraphPad Prism Software version 9.0.2 (San Diego, CA, USA) was used for outlier exclusion with default parameters ROUT (Q=1%).

A ratio between pro-inflammatory and pro-resolutive parameters was calculated to establish a measure for the balance between the pro-inflammatory and pro-resolutive axes of the underlying physiological processes.

A one-tailed *t*-test was used for all statistical comparisons, and *p*-values below 0.05 were rated statistically significant as there was no adjustment for multiple testing. The data presented here are merely explorative and descriptive.

Analyzed Lipids and Lipid Mediators

The following analytes were determined

Poly-unsaturated fatty acids: EPA, DHA, ARA, DPA.

SPM monohydroxylated-containing precursors: 17-HDHA, 18-HEPE, 14-HDHA.

SPMs: Resolvins (rve1, rvd1, rvd2, rvd3, rvd4, rvd5), Maresins (mar1, mar2), Protectins (PD1, PDX), Lipoxins (LXA4, LXB4).

Pro-inflammatory eicosanoid lipid mediators: Prostaglandins (PGE2, PGD2, PGF2α.), Thromboxanes (txb2), Leukotrienes (LTB4).

Results

In this observational study, we observe that the quantification of each parameter was detectable in the sera but not in the same way in the plasma of the participants. All patients showed clinical signs of coughing, dyspnoea, fever, and pneumonia. One patient experienced cerebral thrombosis. Almost all the patients received antibiotic treatment and dexamethasone. All patients received an anticoagulant. Table I depicts the clinical signs and treatment of the patients.

The main aim of this study was the quantification of the Targeted SPM -Eicosanoid lipidomics in human plasma profiles. We measured DHA, ARA, and the EPA metabolome using the “state of the art” targeted LC-MS/MS metabololipidomics.

After quantitation, summation of total ARA-derived pro-inflammatory mediators resulted in a statistically significant increase ($p<0.05$) comparing plasma from patients with

COVID-19 disease or healthy subjects. These pro-inflammatory mediators include LTB_4, PGD_2, PGE_2, PGF_{2a}, and TXB_2, and values altogether were 100-fold times higher than those of healthy subjects (Figure 1A left). Measured prostanoids, including PGD_2, PGE_2, and PGF_{2a}, all together were ~600% increase in plasma from patients infected compared to healthy subjects (Figure 1 middle). TXB_2 was also statistically significantly ($p<0.05$) higher in plasma from patients diagnosed with COVID-19 as compared to healthy subjects, which may reflect that these patients could suffer from coagulopathies (Figure 1 right).

We next studied whether these patients could have a disbalance in SPMs formation (Figure 1

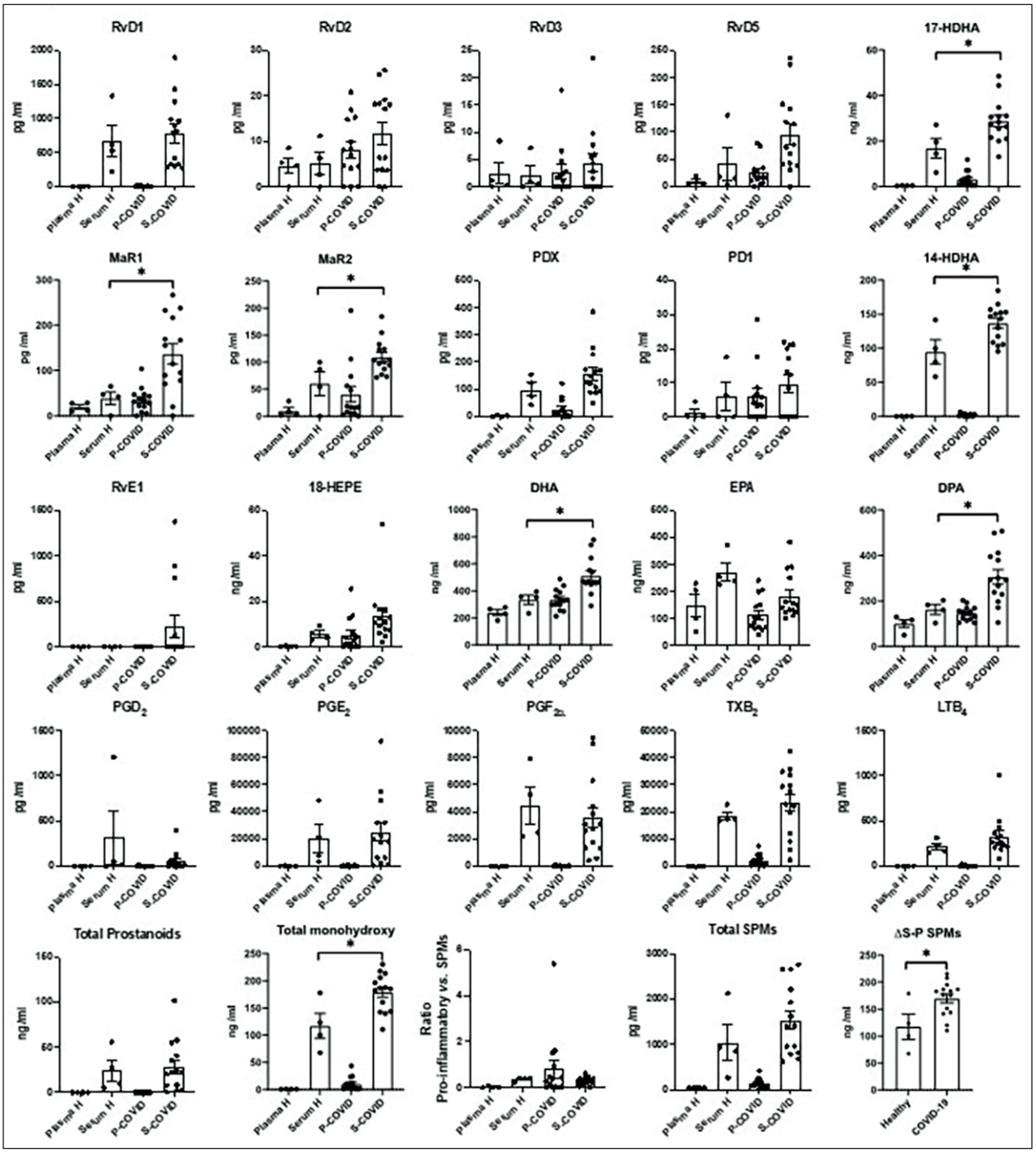

Figure 1. Comprehensive metabololipidomics profile analyses of human plasma(=P) and serum (=S) of healthy controls and SARS COVID-19 patients by LC-MS/MS.

bottom right). Specifically, comparing the ratio of total pro-inflammatory lipid mediators, including LTB_4, PGD_2, PGE_2, PGF_{2a}, and TXB_2 *vs.* Total SPMs formed were statistically significantly different (Figure 1B left). We observed in plasma that the ratio of full pro-inflammatory lipid mediators to the summation of SPMs, including 14-HDHA, 17-HDHA, and 18-HEPA was nominally higher for patients infected with the virus compared to those observed in the plasma of healthy subjects (p=0.07). This finding suggested that infection could impair resolution mechanism(s) due to exacerbated inflammation. Therefore, we quantified the free-fatty-acid-precursors of resolving mediators. We observed that DHA and DPA but not EPA or ARA were statistically significantly higher in infected patients' plasmas (Figure 2A) than healthy subjects. Interestingly, we observed that COVID-19 patients presented nominally higher amounts of the biomarkers monohydroxy-SPMs precursors (i.e., 17-HDHA, from DHA pathway and 18-HEPA from EPA pathway) and statistically significant higher amounts for 14-HDHA derived from the DHA pathway (Figure 2B). This shift in lms suggests that an imbalance between lms may contribute to disease progression, and there is an implied need for pro resolutive mediators.

As shown in Figure 1, the mean value of total prostanoids in the plasma was 10.2 pg/ml in healthy subjects and 65.7 pg/ml in COVID-19 patients (see Figure 1A (middle)). When comparing the differences between the total inflammatory and the thromboxane values between the healthy subjects and the COVID-19 patients, statistically significant differences could also be observed. COVID-19 patients expressed significantly higher values than the healthy controls (see Figure 3B).

The ratio between total pro-inflammatory and total SPM's including 17-HDHA, 14-HDHA, and 18-HEPA was higher in the COVID-19 patient group than in healthy controls.

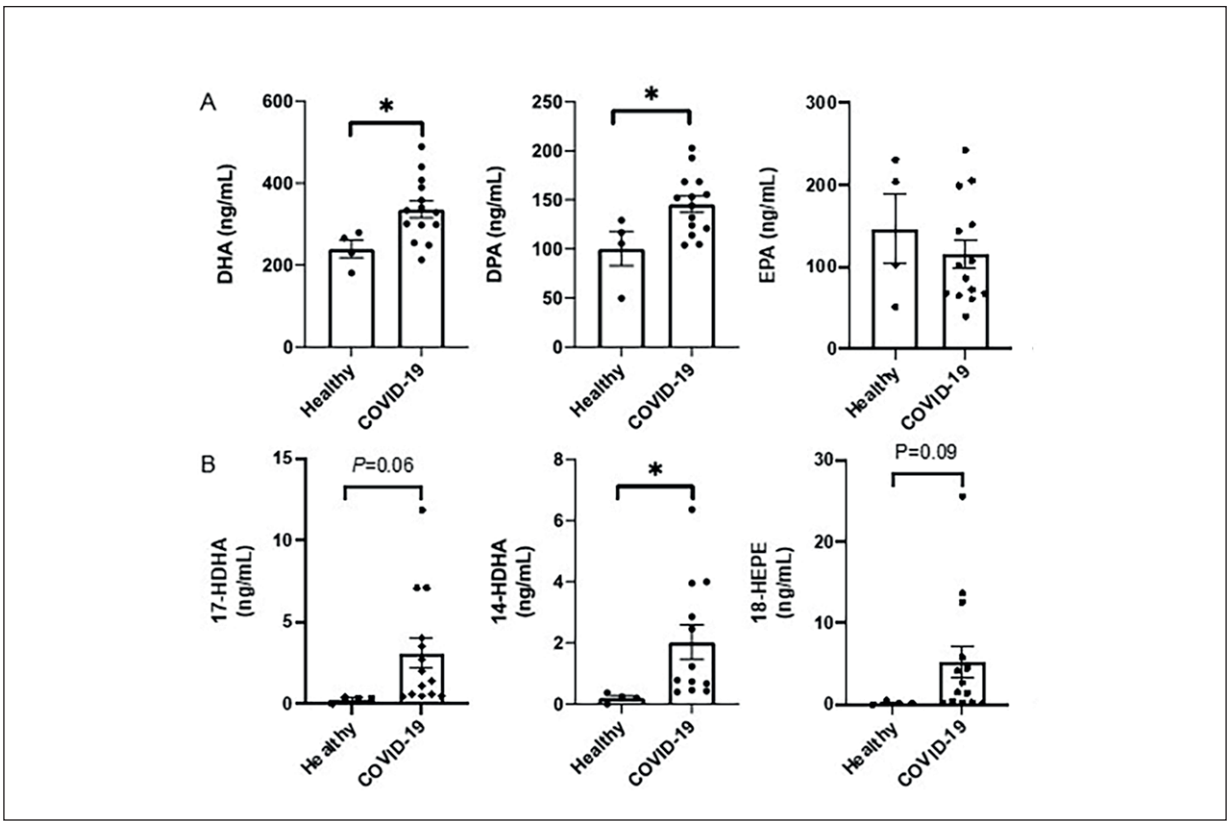

Figure 2. Essential fatty acid mobilization after COVID-19 infection. **A**, DHA, DPA, and EPA levels from human plasma were assessed using LC-MS/MS targeted lipid mediator profiling (ng/mL). **B**, Levels of 17-HDHA, 14-HDHA, and 18-HEPA from human plasma were assessed using LC-MS/MS targeted lipid mediator profiling (ng/mL). The asterisk depicts the statistically significant differences between the healthy controls (n=4) and COVID-19 patients (n=14). Results are expressed as mean ± SEM for indicated n. Comparisons were considered statistically significant when *$p < 0.05$, unpaired one-tailed t-test.

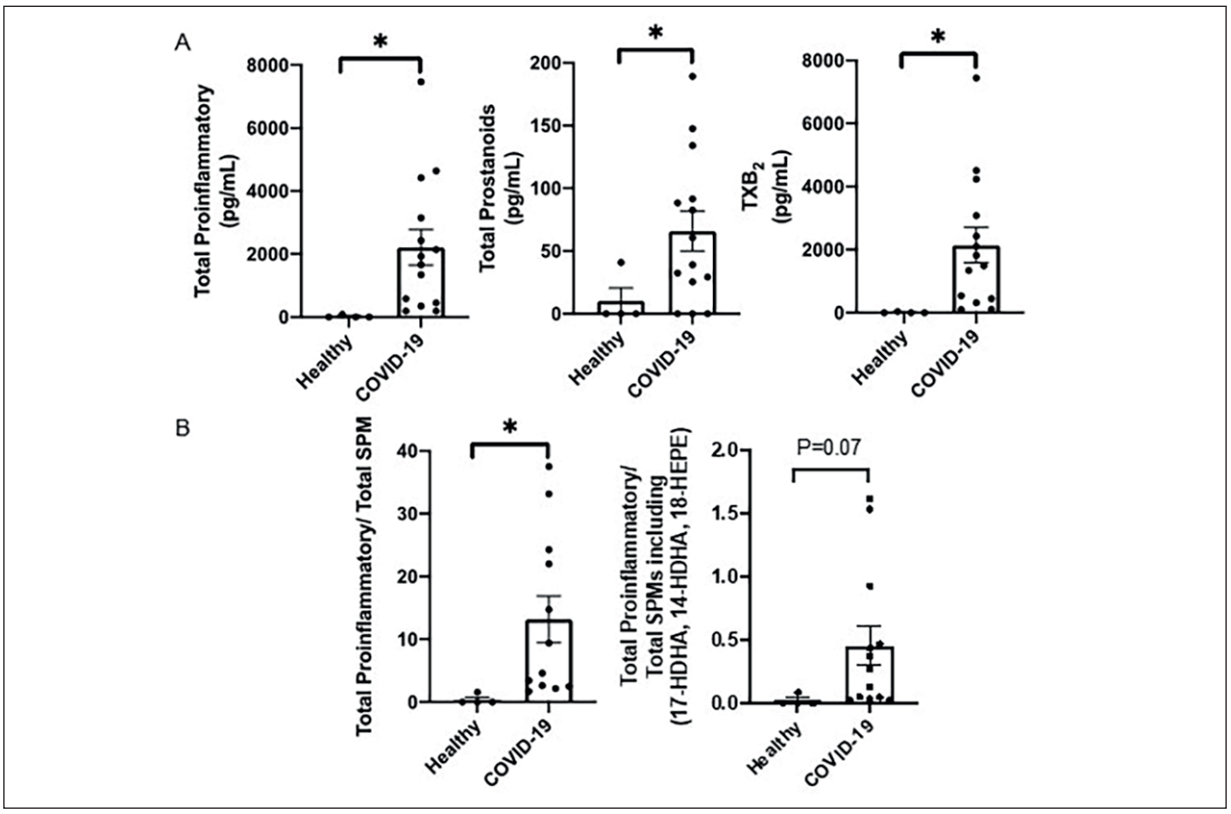

Figure 3. COVID-19 patients present a differential pro-inflammatory lipid mediator profiling compared with healthy controls. **A**, Levels of pro-inflammatory lipid mediators, total prostanoids, and TxB_2 were assessed using LC-MS/MS targeted lipid mediator profiling from human plasma (pg/mL). Monohydroxylated fatty acids including 17-HDHA, 14-HDHA, and 18-HEPE amounts (pg/mL). **B**, The ratio of summation of total pro-inflammatory lipid mediators, including LTB_4, PGD_2, PGE_2, PGF_{2a}, and TXB_2, dived by total SPMs was calculated. Two outliers were discarded using default parameters ROUT (Q=1%) from GraphPad Prism version 9.0.2, GraphPad Software, San Diego, California USA for COVID-19 patients from B left n=4 for healthy group and n=12 for COVID-19 group. Three outliers were discarded (as previously described) in COVID-19 patients from the n=4 healthy group and the n=11 COVID-19 group. Results are expressed as mean ± SEM for indicated n. Comparisons were considered statistically significant when $*p < 0.05$, unpaired one-tailed *t*-test.

Plasma from COVID-19 patients exhibited significantly more eicosanoids than healthy controls. Also, the biosynthesis of SPM's was higher in COVID-19 patients than in the healthy group (Figure 3B).

Next, we accessed serum to determine the ability and the need for these patients to produce SPMs to fight the infection. We assessed LC-MS/MS lipid mediator profiling of human serums from infected and non-infected patients. SPMs were observed in serum from both healthy or infected patients and their precursors or pathway markers. In comparison, serum from infected patients presented a statistically significant number of total SPMs (Figure 3A right) and the summation of their pathway markers 14-HDHA, 17-HDHA, and 18-HEPA (Figure 3A left). Interestingly, the maresins-DHA-derived SPMs mar1 and mar2 were statistically significantly higher in the serum of COVID-19 patients (Figure 4B). Altogether, these results suggest that during COVID-19 disease, there is the biosynthesis of inflammatory ARA-derived mediators and the mobilization of essential pufas for SPMs biosynthesis. The complete lipid mediator profiling for all patient' data is shown in Figure 1. Figures 2-5 present the entire data of the study.

Human plasma or serum was extracted using SPE and subject to targeted LC-MS/MS (see method above). Targeted LM and pathway markers were described. Each mediator was identified using published criteria obtained on their structure, including identification criteria of at least six characteristic diagnostic ions present in their MS-MS spectra. Representative screen captures of MS-MS enhanced product ion (EPI) spectra

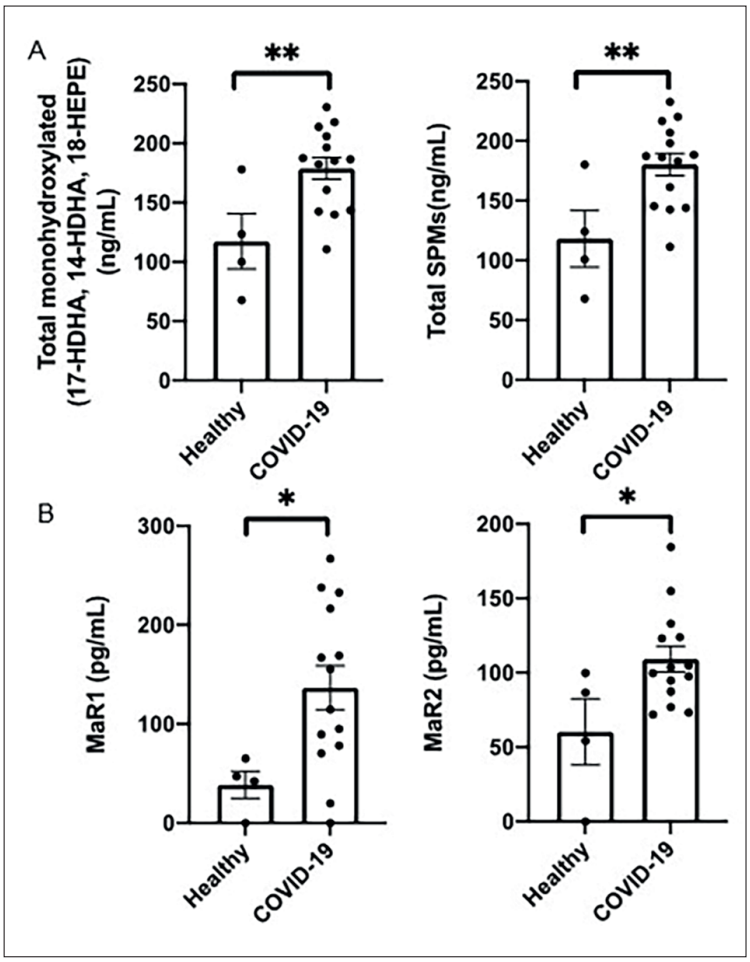

Figure 4. Human COVID-19 serum presents enhanced production of pro-resolving lipid mediators. **A**, Summation of monohydroxylated pathway markers, including 14-HDHA, 17-HDHA, and 18-HEPE (left) together with total SPMs (right), were assessed using LC-MS/MS targeted lipid mediator profiling from human serum (ng/mL). **B**, Quantitation of MaR1 (left) and MaR2 (right) in human serum were assessed using LC-MS/MS targeted lipid mediator profiling (pg/mL). Results are expressed as mean ± SEM for indicated n=4 for healthy group and n=14 for COVID-19 group. Comparisons were considered statistically significant when $*p < 0.05$, $**p<0.01$ unpaired one-tailed *t*-test.

captured from the chromatographic region of (A) LTB4 (B) PGD2 (C) PGE2 (D) PGF2a (E) TXB2 (F) rvd1 (G) PD1 (H) mar1 and (I) mar2. Screen captures were taken using SCIEX OS software. Insets: Chemical structures and prominent fragmentations (Figure 6). Table II depicts all the values in a tabular form.

Discussion

In the present work, a significant difference between the lipid mediator profiles of COVID-19 patients compared to healthy subjects was observed. This disease can lead to a robust inflammatory response, represented by a high abundance of pro-inflammatory signalling molecules like Interleukin-6, C-reactive protein, an increased erythrocyte sedimentation rate, and fibrinogen levels[31].

It was demonstrated by evaluation of LC-MS/MS data that the ratio between (pro-inflammatory) eicosanoid derivatives and pro-resolutive lipid mediator molecules was significantly higher in Sars-cov-2-affected subjects compared to healthy ones. A similar observation was made in a recent work, when the lipidomes of Covid-19 patients with severe symptoms, such as ARDS, were compared to the lipid profiles of only moderately affected patients. In that case, the lipid mediator products of the enzymes ALOX12 and COX2 had been decreased, while those of ALOX5 and cytochrome P450 had been increased[27].

The pro-thrombotic alterations observed in COVID-19 patients may derive from processes initiated by the damage of virus-infected cells. In patients, who require intensive care treatment, high levels of pro-inflammatory cytokines were detected compared to subjects with a moderate manifestation of the disease[17]. Those extensive inflammatory processes may lead to aggravated coagulatory reactions.

An important diagnostic parameter for induction of coagulation is the increase in D-Dimer levels, and for COVID-19, it has become an

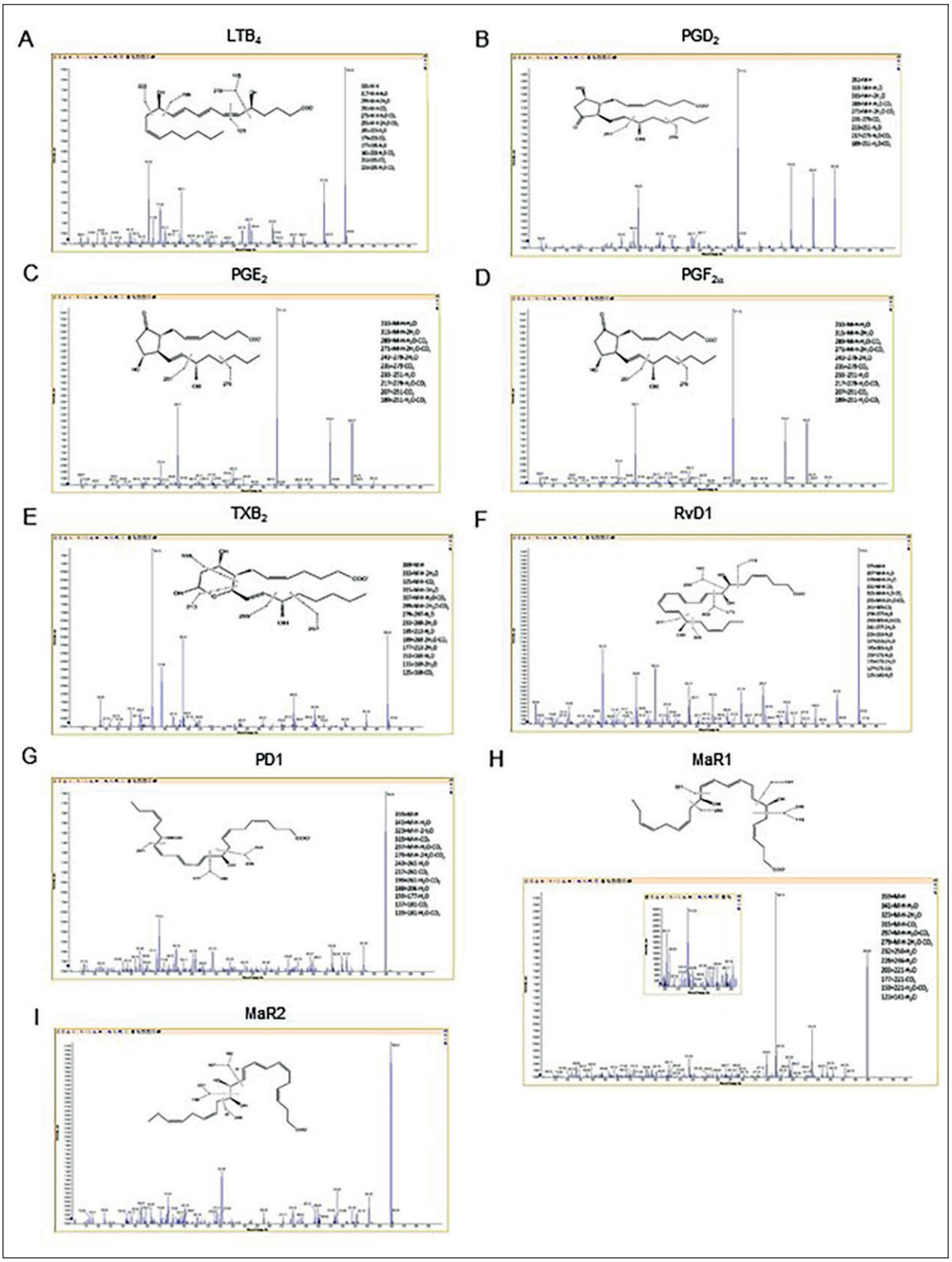

Figure 5. Representative screenshots of LMs analyzed. Representative screenshots of LM were taken using SCIEX OS software; insets indicate lipid mediator fragmentation, and listed ions on the right are included. (**A**) LTB_4 (**B**) PGD_2 (**C**) PGE_2 (**D**) PGF_{2a} (**E**) TXB_2 (**F**) RvD1 (**G**) PD1 (**H**) MaR1 and (**I**) MaR2.

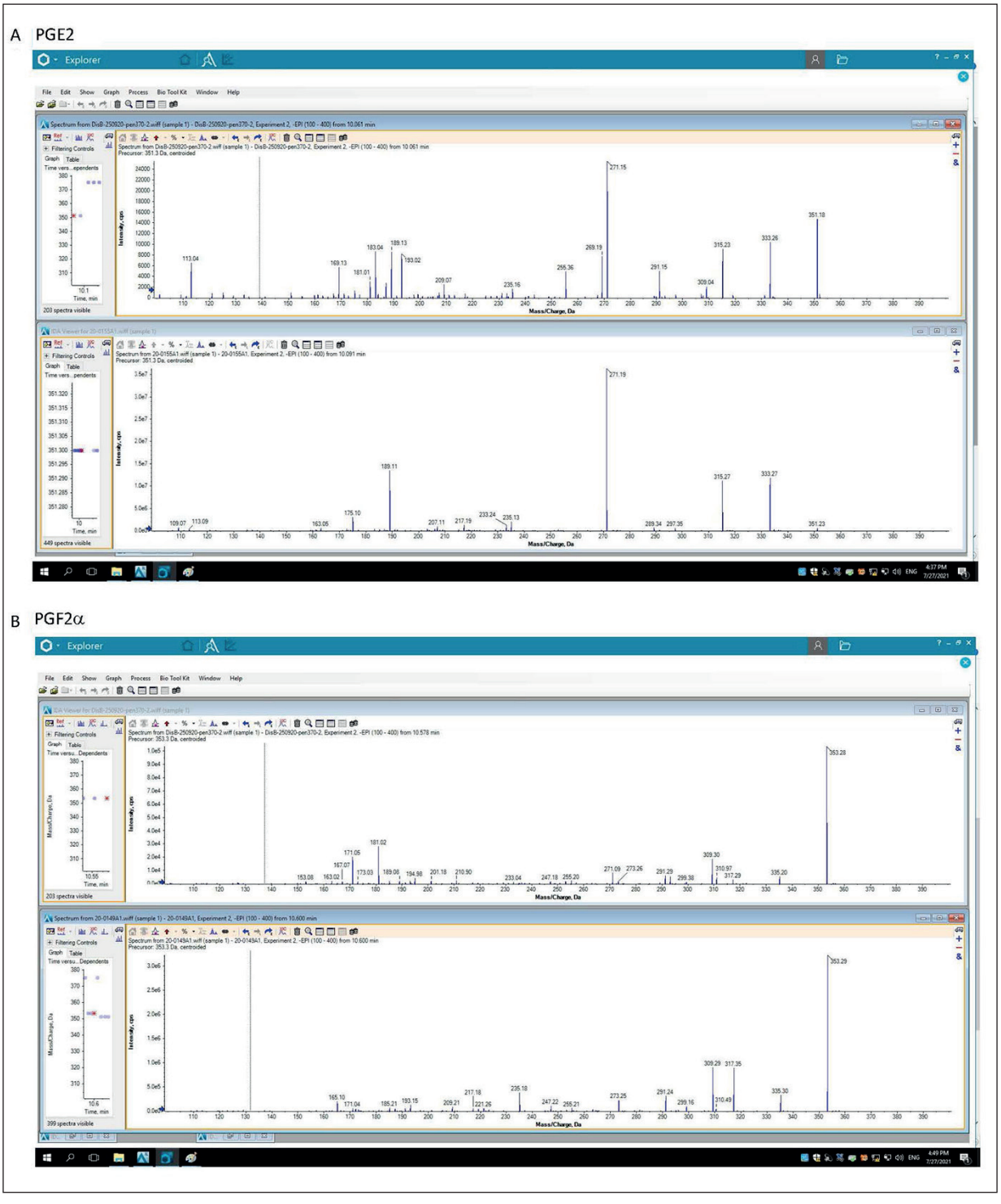

Figure 6 *(Continued).*

indicator for the severity of the disease. Subjects who develop DIC (disseminated intravasal coagulopathy) or asepsis have a high mortality risk[32-34]. The processes leading to these severe coagulopathies are not entirely understood yet. However, the underlying inflammation gives rise to the coagulatory alterations instead of the virus itself.

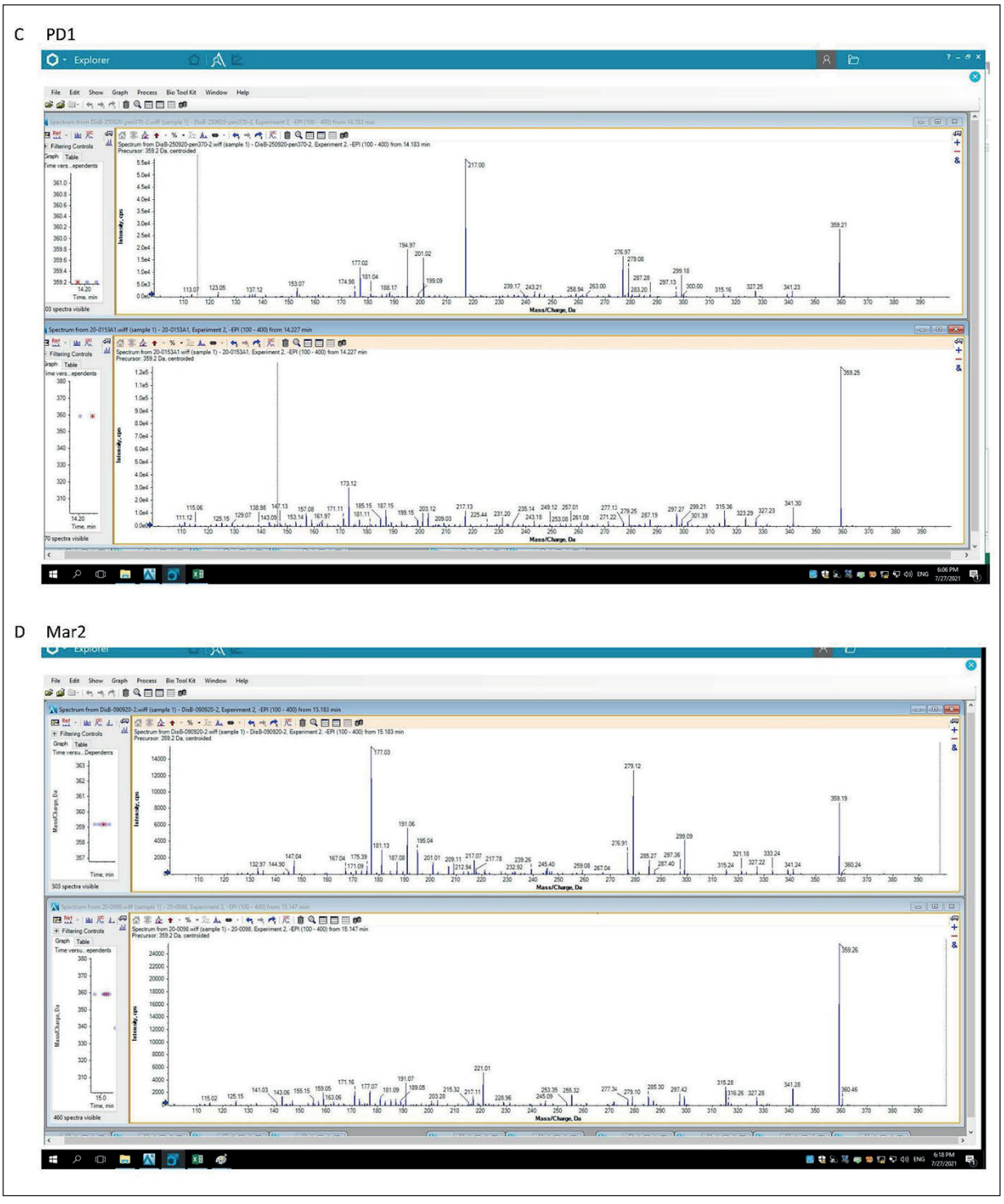

Figure 6. Representative screen captures of LM analyzed. Top panels indicate MS/MS spectra of synthetic lipid mediators used as standard. The bottom panel indicates biological MS/MS spectra of indicated LM. **A**, PGE2, (**B**) PGF2a, (**C**) PD1, and (**D**) MaR2. Blue arrows on Y-axes denote the noise threshold. Screen captures were taken using SCIEX OS software.

The data presented here support this notion: pro-inflammatory parameters of COVID-19 patients were significantly increased compared to healthy persons and, above that, elevated levels of thromboxane TBX2 were detected both in serum and plasma samples of Sars-cov2 -infected sub-

Table II. ARA, DHA, EPA, and DPA metabolome: healthy and COVID-19 lipid mediators amount from human plasma and serum.

	LM levels in human plasma (pg/mL) #(ng/mL)		LM levels in human serum (pg/mL) #(ng/mL)	
	Healthy (n = 4)	COVID-19 (n = 14)	Healthy (n = 4)	COVID-19 (n = 14)
ARA metabolome				
LXA4	–	0.1 ± 0.1	–	–
LXB4	–	0.7 ± 0.5	121.7 ± 121.7	5.2 ± 3.3
LTB4	0.1 ± 0.1	3.2 ± 1.7	215.4 ± 37.4	328.6 ± 59.0
PGD2	–	0.9 ± 0.7	315.9 ± 297.3	55.6 ± 28.2
PGE2	10.2 ± 10.2	46.8 ± 11.2	19947.8 ± 10190.5	25064.7 ± 6893.8
PGF2α	–	18.0 ± 5.8	4470.1 ± 1347.9	3574.2 ± 765.4
TXB2	10.4 ± 10.4	2142.6* ± 561.1	18541.2 ± 1435.6	23425.3 ± 3255.5
ARA#	306.3 ± 127.5	290.6 ± 22.6	439.2 ± 102.3	477.5 ± 35.6
DHA metabolome				
RvD1	2.9 ± 0.9	4.5 ± 1.4	672.3 ± 235.4	783.1 ± 133.6
RvD2	4.6 ± 1.8	8.2 ± 1.8	5.1 ± 2.4	11.8 ± 2.5
RvD3	2.5 ± 2.0	2.8 ± 1.3	2.1 ± 1.7	4.1 ± 1.7
RvD4	0.1 ± 0.1	–	0.3* ± 0.3	–
RvD5	8.5 ± 3.6	25.1 ± 6.4	41.4 ± 29.8	96.2 ± 19.2
17–HDHA#	0.4 ± 0.1	3.1 ± 0.9	16.8 ± 4.4	28.8* ± 2.5
PD1	1.1 ± 1.1	6.0 ± 2.2	5.9 ± 4.1	9.7 ± 2.5
PDX	1.8 ± 1.1	26.5 ± 9.2	98.2 ± 24.7	156.4 ± 23.2
MaR1	19.6 ± 5.0	34.9* ± 7.1	38.6 ± 13.8	136.7* ± 22.2
MaR2	10.2 ± 6.2	40.6* ± 14.6	60.2 ± 22.3	109.3* ± 8.6
14–HDHA#	0.2 ± 0.1	2.2* ± 0.5	94.7 ± 17.7	136.5* ± 7.1
DHA#	239.0 ± 21.9	335.6* ± 20.4	334.6 ± 35.0	511.3* ± 35.5
EPA metabolome				
RvE1	–	–	–	225.4 ± 119.0
18-HEPE#	0.3 ± 0.1	5.3 ± 2.0	5.8 ± 1.4	13.6 ± 3.3
EPA#	147.0 ± 42.3	115.1 ± 16.9	272.8 ± 33.3	182.3* ± 22.9
DPA#	100.3 ± 17.6	145.5* ± 8.3	162.4 ± 21.7	306.3* ± 31.9

Human plasma and serum from healthy and COVID-19 patients were collected as described then were extracted and LM levels investigated using LM-metabolopidomics (see materials and methods for details). Values are express as mean ± SEM, (–) below limit of detection. Healthy *vs*. COVID-19 *$p < 0.05$ unpaired one-tailed *t* test. #(ng/mL).

jects compared to healthy ones (23425.3 ± 3255.5 and 2142.6 ± 561.1 ng/ml compared to 18541.2 ± 1435.6 ng/ml and 10.4 ± 10.4 ng/ml).

On the other hand, Hemorrhagic bleeding disorders are not observed in the context of Sars-cov-2 infections, which contrasts with other single-stranded RNA-viruses, such as Ebola[3]. Also, data from Wuhan support the conception that the inflammatory host response leads to coagulopathies via interlinked signaling pathways.

A comprehensive cohort study demonstrated a relationship between activated neutrophils, platelets, and the dysregulated coagulation cascade that finally led to immunothrombotic damage in various tissues. Utilizing both coagulation tests with peripheral blood samples and histopathological analyses, the scientists identified the systemic hypercoagulability with microvascular thrombosis observed in several organs as characteristic key contributors for severe manifestations of ARDS in COVID-19. Consequently, platelet- and neutrophil count and signs for the coagulation cascade activation were suggested as valuable pharmaceutical targets for the treatment of COVID-19[35]. Therefore, the systematic surveillance of coagulation processes combined with prophylactic anticoagulant treatment has become essential for managing COVID-19- affected patients.

The pure elimination of the infectious agent may not be sufficient to re-establish homeostasis in affected patients. Still, a relatively active cessation of the inflammatory processes and clearing the infection sites is required.

It has been demonstrated in mouse models that thrombi were markedly reduced under the application of the factor resolving D4 (rvd4). The treatment also led to decreased neutrophil infiltration and a higher abundance of monocytes in a pro-resolutive state and cells in the early stages of

apoptosis. Rvd4 also triggered enhanced biosynthesis of further pro-resolutive resolvins of the D-series family. The SPMs, mainly rvd4, were shown to be important modulators of the gravity of thrombo-inflammatory processes while furthering the resolution of thrombi[36].

Elajami et al[37] also showed, in a clinical trial with the primary endpoint in the improvement of de-clotting in coronary arterial disease, that SPMs could be used during long-term conditions and that they can be considered as safe, suggesting that these molecules can also be used in the future in COVID-19 patients.

Interestingly, in patients with coronary arterial disease, the levels of certain pro-resolutive SPMs are reduced compared to healthy subjects. However, when treated with pharmacological doses of EPA and DHA for one year, a clear shift in lipid mediator profile compared to non-treated patients was observed with a decrease in triglyceride levels and pro-inflammatory prostaglandins and a significant decrease increase in certain pro-resolutive SPMs. The SPM-triggered macrophage-based phagocytosis of clots was enhanced in patients treated with the SPM precursors[37].

The results presented here also show different distributions between pro-inflammatory parameters and SPMs in healthy subjects compared to COVID-19 patients. On the other hand, there was no significant alteration between serum and plasma levels of EPA and ARA in patients compared to healthy controls. An increase in DHA may hint at a specific mobilization of the SPM-precursor due to ongoing inflammatory processes. Although there was an increase in the level of certain SPMs in patients suffering from COVID-19, this may not be sufficient to compensate for the immense rise in pro-inflammatory LM observed in these subjects, particularly PG and TBX. The apparent paradox of the concomitant slight decrease of ARA, disregarding the difference in unit range (pg/ml *vs.* Ng/ml), maybe explicable, as the eicosanoid might be an indicator of inflammation in peripheral blood, though being produced at the site of inflammation.

Conclusions

To further shed light on the role of SPMs in COVID-19 disease, it will be informative to investigate the effects of SPM supplementation in clinical trials. This supplementation might be beneficial by preventing the cytokine storm observed in severe manifestations of COVID-19 disease, as the SPMs may enforce the pro-resolutive axis of inflammatory processes. This might also help improving chronic courses associated with inflammation of heart and lung tissue. Also, supplementation with SPMs or their precursor metabolites may improve pathologic conditions for recovered patients or vaccinated subjects. As demonstrated with the present study, the increase in endogenous biosynthesis of SPMs that was observed in sera of COVID-19 patients might be insufficient to compensate for the excessive inflammatory processes observed with the disease, and this relative shortage of pro-resolving lms may result in failed resolution of inflammation. Furthermore, approaches leading to an increase in endogenous SPM production may be beneficial for the course of the disease.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

Pedro Antonio Regidor: Conceptualization, supervision, writing-original draft Xavier de la Rosa: Software; formal analyses; writing-review, and editing Fernando Gonzalez Santos: Project administration, visualization
Jose Miguel Rizo: Project administration, funding acquisition Rafael Gracia Banzo. Resources Rafael S Silva: Investigatio.

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