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Resolvin E1-Chemerin receptor 1 axis is dysregulated in critical COVID-19 patients

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ABSTRACT

Resolvin E1 (RvE1) and Resolvin D1 (RvD1) are key resolvins implicated in inflammation resolution of respiratory and infectious diseases. In contrast to cytokines, they have been scarcely explored in COVID-19 and their ability for discriminating COVID-19 severity and patient outcomes has not been compared with that of cytokines. Therefore, among a panel comprising cytokines (interleukin (IL)-1beta, IL-6, IL-10, tumor necrosis factor alpha, interferon gamma and granulocyte-macrophage colony-stimulating factor), RvD1 and RvE1 and their respective receptors (FPR2, Chemerin$_1$), we evaluated which mediators better distinguished COVID-19 severity, the need of mechanical ventilation and patient mortality.

Blood was collected from 61 patients with "severe" ($n = 27$), "critical" ($n = 17$) and "critical on veno-venous extracorporeal membrane oxygenation (VV-ECMO)" (n = 17) COVID-19 at admission, days 3–4 and days 5–8,

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and from controls ($n = 23$) at a single time point. We measured cytokines by multiplex immunoassays, resolvins by enzyme-linked immunosorbent assays, and FPR2 and Chemerin$_1$ mRNA by real-time quantitative polymerase chain reaction.

We obtained principal component analysis (PCA)/partial least squares discriminant analysis (PLS-DA) models significantly differentiating ($P < 0.001$): controls from each patient group; "severe" from all critical patients; patients without or with mechanical ventilation, and survivors from non-survivors. RvE1 consistently showed a variable importance in projection (VIP) score > 2.5 and a p(corr) >0.8, being the most relevant discriminating variable. Univariate and repeated measures multivariate analyses showed higher RvE1 in "critical on VV-ECMO", mechanically ventilated patients and non-survivors, while Chemerin$_1$ exhibited an opposite profile. RvE1 positively correlated with inflammation and partial pressure of CO_2, whereas Chemerin$_1$ correlated with lower inflammation, better respiratory function and lower hospital length of stay.

We conclude that RvE1 was the mediator best distinguishing COVID-19 severity and that RvE1-Chemerin$_1$ axis is dysregulated in this disease.

1. Introduction

Since the onset of the most recent pandemic in 2020, significant progress has been made in understanding COVID-19, a human disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Although it causes asymptomatic infection in a relevant percentage of infected people, SARS-CoV-2 is also responsible for severe and critical clinical conditions, the latter requiring life-sustaining therapies such as mechanical ventilation or even veno-venous extracorporeal membrane oxygenation (VV-ECMO) [2]. Currently, it is well established that inflammatory dysregulation plays a central role in the pathophysiology of COVID-19 [3], particularly in the critically ill [4]. These dysregulated inflammatory responses frequently involve a cytokine storm, endothelial activation and thrombosis, primarily within the pulmonary vasculature, but later expanding to other organs, leading to extrapulmonary manifestations [5]. This cytokine storm is characterized by the overproduction of cytokines, such as interleukin (IL)-1β, IL-2, IL-6, IL-8, IL-10, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF) [6,7], and some of them have been associated with COVID-19 severity and mortality, and even with long COVID-19 [8–10]. Therefore, targeting the cytokine storm mediators arose as a potential therapeutic strategy to reduce COVID-19 severity and mortality rates [11]. However, although several anti-inflammatory and immunomodulatory drugs have been initially proposed as therapeutic agents, current guidelines only recommend the use of corticosteroids, IL-6 receptor blockers and Janus kinase inhibitors in patients with severe or critical COVID-19 [12]. Furthermore, reflecting the broad immunological dysregulation, other biomarkers have been proposed as markers of COVID-19 severity, including immune checkpoint molecules such as programmed cell death-1 (PD-1) and PD-1 ligand 1, which limit the excessive activation of the host immune system to infections, but may also lead to immune exhaustion when overexpressed [13]. Additionally, other hematological indices like the neutrophil-to-lymphocyte ratio (NLR) and the neutrophil-to-monocyte ratio (NMR) have been found elevated in critically ill patients, indicating a disturbance in the balance between immune cells, where the innate immune proinflammatory response predominates over an insufficient adaptive immunity [14,15].

In contrast to the plethora of studies evaluating cytokines in COVID-19, little attention has been given to resolution of inflammation pathways in this disease. Once thought to be a passive process, the resolution of inflammation has emerged as a crucial part of the inflammatory response [16]. It is orchestrated by a superfamily of lipid mediators (e.g., lipoxins, resolvins, protectins and maresins) derived from essential fatty acids and known as specialized proresolving mediators (SPMs), as well as by proteins (e.g., annexin A1) and other compounds, which act in an active and temporally organized manner [17,18]. The evidence of SPMs potential benefits in various respiratory and infectious diseases [19,20] led some research groups to investigate the resolution status in COVID-19 patients, in whom they found altered profiles of lipid mediators, consistent with disrupted resolution mechanisms [21–24]. Moreover, this dysregulation appears to escalate with disease severity [25–28].

Resolvin D1 (RvD1) and resolvin E1 (RvE1) were the first identified members of the D-series and E-series resolvins, derived from the omega-3 polyunsaturated fatty acids (PUFA) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), respectively [29]. Since then, additional members of their families (RvD2–6 and RvE2–4) have been discovered, as well as DHA-derived cysteinyl-SPMs, and docosapentaenoic acid (DPA)-derived 13-series resolvins [30–32]. However, RvD1 and RvE1 remain the most extensively studied resolvins and the only ones in the IUPHAR/BPS Guide to Pharmacology with recognized interactions with various G protein-coupled receptors (GPCRs), through which they elicit their anti-inflammatory and proresolving actions [33–35]. RvD1 binding to FPR2 receptor (FPR2) (formerly named FPR2/ALX or ALX), which also serves as a receptor for lipoxin A_4 and annexin A1, and *GPR32* receptor has been recognized by many research groups [36–41]. However, some controversy remains as certain reports were unable to confirm these interactions, such as the work from Alnouri et al. that also suggests that DHA-derived SPMs act through allosteric modulation of prostaglandin EP_4 receptor [42]. RvE1 is recognized as the most potent endogenous ligand of chemerin receptor 1 (Chemerin$_1$), formerly known as ChemR23 [35]. Indeed, Chemerin$_1$ has been shown to mediate RvE1 protective effects in several studies from independent groups [43–46], although there is also contradictory literature that failed to confirm these findings [47,48]. Additionally, RvE1 has also been suggested as a partial agonist of the leukotriene B_4 receptor, BLT_1 [29,48]. Both RvD1 and RvE1 evoke potent bioactions on different cell types, including neutrophils, macrophages, dendritic cells and platelets, that have proven to be protective in infectious and respiratory disease models [33,49]. The administration of RvD1 enhanced the effectiveness of antibiotics in clearing bacteria [50] and reduced lung infection and inflammation in a cystic fibrosis mice model [51] and in an acute lung injury model [52]. Likewise, RvE1 was reported to promote the resolution of inflammation and bacterial removal in acute lung injury models [53,54], improve alveolar fluid clearance in LPS-induced acute respiratory distress syndrome (ARDS) [55] and dampen airway inflammation in allergic asthma models [56–58]. Besides, its receptor Chemerin$_1$ was shown to be crucial in stimulating antiviral immunity and reducing lung inflammation in a viral pneumonia model [59]. In humans, RvE1 was found to be increased in the plasma of non-surviving sepsis patients [60]. However, the reports on resolvins profiles among COVID-19 patients are inconsistent [21–23,26,28,61] and none of these studies investigated whether SPMs are better discriminators of disease severity than cytokine mediators.

Given the significant focus on cytokines and the limited clinical benefits of therapeutic strategies targeting them, we recognize the need to investigate resolution pathways to strengthen the evidence supporting future resolution-based therapies. We aimed at evaluating a biomarker panel comprising systemic cytokines, as well as resolvins D1 and E1 and their receptors, in order to identify which mediators best distinguish between: (1) healthy controls and hospitalized COVID-19 patients; (2) severe and critically ill COVID-19 patients; (3) COVID-19

patients requiring or not mechanical ventilation; and (4) survivors and non-survivors. Additionally, for the best group discriminator, we assessed its correlations with inflammation, respiratory function and hospital length of stay in all patients.

2. Material and methods

2.1. Study design and population

The present study is part of a larger research project (RESEARCH 4 COVID-19 grant, project 519-reference number 613690173, "Unresolved inflammation and endothelitis in severe COVID-19 patients: identification of risk stratification biomarkers and therapeutic targets", FCT – Fundação para a Ciência e a Tecnologia) involving patients from the ward of the Service of Infectious Diseases and from the intensive care units (ICU) of the Service of Intensive Care Medicine and the Service of Infectious Disease of a tertiary hospital (Centro Hospitalar Universitário São João, CHUSJ). From September 2020 to February 2021, sixty-one patients ($n = 61$) of both sexes who were hospitalized in the context of hypoxemic respiratory failure and symptomatic for >1 day were consecutively enrolled in this single-center cohort study, after a laboratory-confirmed diagnosis of SARS-CoV-2 infection, defined by a positive result on a RT-PCR assay of a specimen collected on a nasopharyngeal swab. Most patients were recruited within 72 h of a positive RT-PCR result. Patients were excluded if they were under 18 years of age, pregnant or lactating, or had a history of vasculitis or connective tissue disease. Admission to the ward or ICU and decision and time for intubation, mechanical ventilation and/or VV-ECMO was based on clinical judgement according to "lege artis". Patients were divided into two groups according to COVID-19 disease severity [2]: patients with severe COVID-19 ($n = 27$) not requiring intensive care treatment and admitted to the ward, and patients with critical COVID-19 ($n = 34$) admitted to the ICU. The group of patients with critical COVID-19 was further subdivided into two groups based on the need for VV-ECMO support: group of critically ill COVID-19 patients without VV-ECMO support (critical COVID-19, $n = 17$) and group of critically ill COVID-19 patients on VV-ECMO (critical COVID-19 on VV-ECMO, n = 17), as shown in supplementary data (Fig. S1). Severe COVID-19 was characterized by the presence of oxygen saturation < 90 % on room air, signs of pneumonia or signs of severe respiratory distress, whereas critical disease was defined as patients presenting criteria for ARDS, sepsis, septic shock, or other conditions that require life-sustaining therapies, according to the World Health Organization's guidelines [2]. Due to the prospective nature of our sampling, we were able to capture a heterogenous population of ward patients and ICU patients. All eligible patients provided written informed consent. For ICU patients unable to give consent, this was solicited to their next of kin and, when possible, these patients provided informed consent retrospectively. Controls ($n = 23$) were recruited among healthy blood donor volunteers from the Service of Immunohemotherapy of CHUSJ before the COVID-19 pandemic, who gave oral informed consent to participate as controls in studies involving critical care patients. The study was conducted in accordance with the Guidelines for Good Clinical Practice and the 1975 Declaration of Helsinki after approval by the CHUSJ Health Ethics Committee [CES 75–16, with project amended specifically for inclusion of subjects with COVID-19, within the scope of a RESEARCH 4 COVID-19 grant from FCT (special support for rapid implementation projects for innovative response solutions to COVID-19 pandemic)]. CES 75–16 approval did not included access to the clinical records of controls (blood donor volunteers), only their sex and age.

2.2. Clinical data and sample collection

The medical team of the project followed all patients during their stay in the ward or ICU and assessed relevant clinical and demographic parameters for each patient. This data, along with routine laboratory data, were anonymously coded to the project database, guaranteeing confidentiality. Illness severity was assessed by the Acute Physiology and Chronic Health Evaluation II (APACHE II) and Simplified Acute Physiology Score II (SAPS II) scoring systems at ICU admission. Total hospital length of stay and ICU length of stay were also evaluated. These calculations considered not only hospitalization at CHUSJ but also at other hospitals where some patients had initially been admitted before transfer to CHUSJ, or to which they were subsequently transferred after their stay at CHUSJ. Therefore, total hospital length of stay was defined as the entire consecutive period of hospitalization, including both ward and ICU stays, whereas ICU length of stay included only the total period spent in ICU. In fact, as a tertiary hospital, CHUSJ was highly requested during the pandemic for specialized care, requiring strict space management. Consequently, patients were frequently transferred to hospitals in their residential area once they no longer required tertiary-level care. Moreover, the group of patients with critical COVID-19 on VV-ECMO included eleven patients who were previously hospitalized in the ICU of other hospitals before being transferred to the ICU of CHUSJ for VV-ECMO cannulation, as it is recognized as a reference center for ECMO in Portugal. Also, all patient groups included a few patients that were further transferred from CHUSJ to other hospitals and all consecutive period of hospitalization was counted for calculation of total hospital length of stay. Mortality within 1-year post-hospital discharge was assessed through clinical records review or by telephone contact when this information was not available.

For all patients, blood samples were collected at three time points throughout their first week of hospitalization at CHUSJ, whenever possible: up to 48 h (days 1–2; admission), on days 3–4 and on days 5–8 after admission. All sample collections from critical COVID-19 patients on VV-ECMO were started after VV-ECMO initiation. Samples (blood) from controls were collected at a single time point. All samples were processed within 1–2 h of collection and stored at −80 °C until assayed.

2.3. Quantification of routine markers

All the routine laboratory analyses were performed at the Service of Clinical Pathology of CHUSJ. Quantification of lactate, partial pressure of oxygen (PaO_2) and partial pressure of carbon dioxide ($PaCO_2$) was performed by arterial blood gas analysis. Fraction of inspired oxygen (FiO_2) was obtained from oxygen administration device and oxygen dose information in the medical records and the PaO_2/FiO_2 ratio was calculated. Differential leukocyte count (leukocytes, neutrophils, eosinophils, monocytes and lymphocytes) was analyzed by flow-cytometry in an automated hematology analysis system (Sysmex 5000; Emílio de Azevedo Campos, Porto, Portugal), with subsequent calculation of neutrophil-to-lymphocyte ratio (NLR) and neutrophil-to-monocyte ratio (NMR). Since we did not have permission to access the hospital laboratory reports of the control group, their routine clinical biomarkers were not included in this study.

2.4. Quantification of resolvin D1 and resolvin E1

Serum resolvin D1 (s-RvD1) and serum resolvin E1 (s-RvE1) were measured by enzyme-linked immunosorbent assays (ELISA) using the commercial kits "Human Resolvin D1 (RvD1) ELISA kit, Cat.No: MBS756429" and "Human Resolvin E1 (RvE1) ELISA Kit, Cat.No: MBS025958", respectively, from MyBioSource, Inc., San Diego, California, USA. These assays were performed in unextracted samples since the manufacturer did not recommend prior solid-phase extraction. The manufacturer reported that Human Resolvin D1 kit did not show cross-reactivity with the analogues RvD2, RvD4 and RvD6. Regarding the Human Resolvin E1 kit, no cross-reactivity was found with the analogues RvE2 or with RvD1, RvD2 and RvD3.

2.5. Relative quantification of FPR2 and Chemerin$_1$

FPR2 and Chemerin$_1$ were quantified by Real-Time quantitative Polymerase Chain Reaction (RT-qPCR). Total RNA was extracted from peripheral blood mononuclear cells (PBMCs) using NZY Total RNA Isolation kit (NZYTECH, Lisbon, Portugal) according to the manufacturer's instructions. RNA quantification was performed using Nanodrop 2000 (ThermoFisher Scientific, Waltham, Massachusetts, USA), and the RNA integrity was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, California, USA). The NZY First-STRAND cDNA Synthesis Kit (NZYTECH, Lisbon, Portugal) was used to reverse transcribe 500 ng of total RNA with random primers, and the resulting cDNA was diluted 1:20, aliquoted and stored at 4 °C for subsequent use. The expression levels of the selected genes were measured by RT-qPCR using the StepOnePlus Real-Time PCR System (Applied Biosystems, ThermoFisher Scientific, Waltham, Massachusetts, USA) under a 3-step program: denaturation at 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min and finished with a melt curve stage of 95 °C for 15 s, 60 °C for 1 min and an increase of 0.5 °C/s to 95 °C. Each reaction was performed in triplicate, with SensiFAST SYBR Hi-ROX Kit (Bioportugal, Porto, Portugal), 400 nM of primers and 3 μL of 20× diluted cDNA (described above), in a 12 μL final volume. A standard curve made up of one-half serial dilutions of pooled cDNA of a representative number of samples from all groups was run on each plate for each primer set for quantification. Target gene expression was normalized to the expression of *GAPDH*. The following primer sequences were used for detecting transcripts: *GAPDH*, F: 5'-CCATCACCATCTTCCAGGAG-3′, R: 5'-GCATGGACTGTGGTCATGAG-3′; *FPR2*, F: 5'-AGCCCCAACTAATGACACGG-3′, R: 5'-TGACCCCATCCTCACATTGC-3′; *CMKLR1*, F: 5'-CATCATCAGCTCTGACCGCT-3′, R: 5'-TGTCCCGGAAGACGAGAGAT-3′.

2.6. Quantification of cytokines

Serum proinflammatory cytokines (s-TNF-α, s-IL-1β, s-IL-6) and dual role cytokines (s-IL-10, s-IFN-γ and s-GM-CSF), were evaluated by multiplex immunoassays using a Luminex 200 analyzer (Luminex Corporation, Austin, TX, USA), according to the protocols of MILLIPLEX® MAP Human High Sensitivity T Cell Magnetic Bead Panel (Millipore Corporation, Billerica, MA, USA). Raw data analysis (mean fluorescence intensity) was performed using ISTM 2.3 software (Luminex Corporation, Austin, TX, USA).

2.7. Statistical analysis

Results of univariate analyses are expressed as mean ± standard error of the mean (SEM) or as median (25th percentile; 75th percentile) for data with normal or non-normal distribution, respectively, or as percentage, and are graphically represented as Box and Whiskers plots. Statistical analysis was conducted using the GraphPad Prism 10 software (La Jolla, USA). Results were analyzed by unpaired Student's *t*-test for parametric data or Mann–Whitney *U* test for nonparametric data, for comparisons between two groups. When comparing three or more groups, one-way ANOVA followed by a Tukey's multiple comparison test was used for parametric data, while a Kruskal-Wallis test followed by a Dunn's post hoc test was applied for nonparametric data. Categorical variables were analyzed by the Chi Square test. We used Spearman's correlation analysis to estimate correlations between sets of nonparametric data among all patients at admission. P values <0.05 were considered significant.

Multivariate analyses using principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) were performed using a dataset consisting of 10 variables (s-TNF-α, s-IL-1β, s-IL-6, s-IL-10, s-IFN-γ, s-GM-CSF, s-RvE1, s-RvD1, FPR2 and Chemerin$_1$) and a number of observations (N) that differ according to each model, referring to all the blood samples analyzed. Data was mean-centered and pareto-scaled before statistical analysis. PCA was used to differentiate the groups, whilst PLS-DA was performed to identify the variables contributing most to the observed separation considering a variable importance in projection (VIP) score > 1.5 and a correlation coefficient (p(corr)) $> |0.8|$ [62,63]. Score scatter plots and p(corr) vs VIP plots were constructed for each model. SIMCA 14 software (MKS Umetrics, Umeå, Sweden) was used to perform PCA and PLS-DA models.

Repeated measures multivariate analyses, using the IBM SPSS Statistics 27 software (IBM Corporation, New York, USA), were conducted to examine the relationship between s-RvE1 or Chemerin$_1$ (dependent variables) and the COVID-19 patient group, mechanical ventilation and mortality within 1-year post-hospital discharge, adjusted for age and sex, among all patients during the first week of hospitalization.

To prevent possible bias in clinical evaluation, all the patients were examined by the same medical team included in the project. To ensure comparability of biomarkers assessment, samples from controls, severe COVID-19, critical COVID-19 and critical COVID-19 on VV-ECMO groups were evenly distributed in each assay plate. There were missing values in some biomarkers due to insufficient volume of samples or reagents to perform sample processing, dilution tests and assays. We had no permission to measure routine clinical biomarkers in controls (blood donor volunteers), or to have access to their hospital laboratory reports. The final number per group for the biomarkers/parameters evaluated at admission is shown in supplementary data (Table S1). In addition, the number of patients and samples collected decreased throughout the first week of hospitalization due to patients' death, withdrawal of consent or hospital discharge and due to medical/nurse team logistics at a specific time of collection, as described in supplementary figure (Fig. S1). To avoid biasing the results, no imputation for missing values was used.

Sample size was defined according to the primary objectives of our FCT funded RESEARCH 4 COVID-19 project that consisted in characterizing resolution of inflammation and endotheliitis. Based on preliminary evaluations of specialized proresolving mediators in healthy controls and patients with severe and critical disease, using power analysis, we calculated a sample size of 21 subjects per group to obtain an 80 % power, at a 5 % significance level (effect size-to-standard deviation ratio ca. 0.9). Since there was an elevated number of critically ill patients on VV-ECMO and a high heterogeneity of values between critically ill patients without VV-ECMO support vs those on VV-ECMO, we further divided the group of patients with critical COVID-19 into two groups: critically ill (without VV-ECMO) and critically ill on VV-ECMO. Despite this change, we had a total sample size of 84 subjects (i.e. 4 times the 21 initially estimated), albeit with only 17 patients per group in the two critically ill groups. Data concerning demographic, clinical and biochemical parameters, including proinflammatory cytokines, have been recently used by our group as part of studies on the same cohort to evaluate their association with other biomarkers [64,65]. Reporting of the study conforms to STROBE statement along with references to STROBE and the broader EQUATOR guidelines [66].

3. Results

3.1. Population demographic and clinical characterization

Demographic and clinical characteristics of the subjects included in the study are presented in Table 1.

Severe COVID-19 patients were significantly older than controls ($P < 0.01$), whilst critically ill COVID-19 on VV-ECMO patients were significantly younger than severe and critically ill COVID-19 patients ($P < 0.001$ and $P < 0.05$, respectively). There were no significant sex differences between groups, but men were predominant in all groups. Similarly, no significant sex differences were observed between mechanically ventilated and non-mechanically ventilated patients or between survivors and non-survivors (supplementary data, Table S2). Regarding comorbidities, arterial hypertension was the most prevalent

Table 1
Demographic and clinical characterization at admission and follow-up parameters of the study population.

Demographic and clinical parameters	Controls ($n = 23$)	Severe COVID-19 (n = 27)	Critical COVID-19 (n = 17)	Critical COVID-19 on VV-ECMO (n = 17)	*P* value
Age (Years)	57 (53; 63)	71 (63; 80)**	67 (55; 72)	55 (40; 59)$^{\#\#\#,\$}$	<0.001
Sex: Men, n (%)	15 (65)	17 (63)	11 (65)	11 (65)	0.999
Sex: Women, n (%)	8 (35)	10 (37)	6 (35)	6 (35)	0.999
Comorbidities, n (%)					
Diabetes	n.d.	11 (41)	6 (35)	4 (24)	0.502
Obesity	n.d.	7 (26)	8 (47)	10 (59)	0.081
Arterial Hypertension	n.d.	18 (67)	13 (76)	8 (47)	0.188
Heart Failure	n.d.	6 (22)	3 (18)	1 (6)	0.357
Respiratory Disease	n.d.	8 (30)	4 (24)	2 (12)	0.389
Renal Disease	n.d.	6 (22)	4 (24)	0 (0)	0.099
Malignancy	n.d.	2 (7)	0 (0)	0 (0)	0.272
APACHE II Score	n/a	n/a	17 ± 2	19 ± 2	0.423
SAPS II Score	n/a	n/a	42 ± 4	40 ± 4	0.666
Therapeutics at Admission, n (%)					
Dexamethasone	n/a	21 (78)	16 (94)	16 (94)	0.172
Remdesivir	n/a	1 (4)	0 (0)	2 (12)	0.263
Antibiotics	n/a	5 (19)	7 (41)	9 (53)	0.051
PaO_2/FiO_2 ratio	n/a	257 (230; 287)	92 (68; 137)$^{\#\#\#}$	100 (76; 119)$^{\#\#\#}$	<0.001
$PaCO_2$ (mmHg)	n/a	32 ± 1	37 ± 1$^{\#}$	48 ± 2$^{\#\#\#,\$\$\$}$	<0.001
Lactate (mmol/L)	n/a	1.1 (1.0; 1.6)	1.5 (1.1; 1.8)	1.5 (1.2; 1.7)	0.258
Follow-up					
Type of Oxygen Support During Hospitalization, n (%)					
Mechanical Ventilation	n/a	2 (7)	11 (65)	17 (100)	<0.001
NIV	n/a	5 (19)	11 (65)	14 (82)	<0.001
High-Flow Cannula	n/a	9 (33)	13 (76)	9 (53)	0.020
Supplementary Oxygen	n/a	26 (96)	13 (76)	16 (94)	0.081
ICU length of stay (days)	n/a	0 (0; 0)	16 (7; 33)$^{\#\#\#}$	34 (16; 74)$^{\#\#\#}$	<0.001
Total Hospital length of stay (days)	n/a	7 (5; 15)	22 (11; 57)$^{\#\#}$	43 (25; 116)$^{\#\#\#}$	<0.001
Mortality within 1-year post-hospital discharge n (%)	n/a	4 (15)	4 (24)	4 (25)	0.697

APACHE II, acute physiology and chronic health evaluation II; FiO_2, fraction of inspired oxygen; ICU, Intensive Care Unit; n/a, not applicable; n.d., not determined; NIV, non-invasive ventilation; $PaCO_2$, partial pressure of arterial carbon dioxide; PaO_2, Partial pressure of arterial oxygen; SAPS II, Simplified Acute Physiology Score II; VV-ECMO, veno-venous extracorporeal membrane oxygenation; Results are expressed as number (%), mean ± SEM or as median (25th percentile; 75th percentile) for data with normal or non-normal distribution, respectively. $^{**}P < 0.01$ vs Controls; $^{\#}P < 0.05$ vs Severe; $^{\#\#}P < 0.01$ vs Severe; $^{\#\#\#}P < 0.001$ vs Severe; $^{\$}P < 0.05$ vs Critical; $^{\$\$\$}P < 0.001$ vs Critical.

in severe and critically ill COVID-19 patients, while obesity was the most prevalent in critical COVID-19 on VV-ECMO patients, although no significant differences were found between patient groups. There were no differences in APACHE II and SAPS II scores between the groups of critically ill patients.

At admission, almost all patients initiated the treatment with dexamethasone and very few with remdesivir, with no differences between groups. There was a tendentially higher proportion of critically ill COVID-19 patients (with or without VV-ECMO) receiving antibiotics compared to severe patients ($P = 0.051$).

Lactate concentrations at admission did not differ between patient groups. However, $PaCO_2$ was significantly increased in critical patients, especially in those with VV-ECMO, and the PaO_2/FiO_2 ratio was significantly lower in both groups of critically ill patients compared to those with severe COVID-19 ($P < 0.001$). Accordingly, there was a higher need for mechanical ventilation, non-invasive ventilation and high-flow cannula oxygen in all critical COVID-19 patients (with or without VV-ECMO) when compared with severe COVID-19 patients ($P < 0.001$, $P < 0.001$ and $P = 0.02$ respectively).

Both groups of critically ill patients had a longer length of stay in the ICU than severe patients ($P < 0.001$), because only five severe COVID-19 patients needed a temporary upgrade of care to ICU in the first week of hospitalization [median ICU length of stay: 11 (3; 36) days]. At ICU admission, those five patients had mean APACHE II and SAPS II scores of 11 ± 1 and 28 ± 4, respectively. Also, although not statistically significant, patients with critical COVID-19 on VV-ECMO had a longer length of stay in ICU than patients with critical COVID-19. Furthermore, both groups of critically ill patients had a longer total hospital length of stay when compared to severe COVID-19 patients ($P < 0.001$ and $P < 0.01$, respectively). There were no significant differences in mortality within 1-year post-hospital discharge between COVID-19 patient groups.

3.2. Inflammatory and resolutive profiles at admission

Inflammatory and resolutive profiles at admission of the study population are presented in Table 2. At admission, proinflammatory cytokines were significantly increased in all COVID-19 patient groups compared to controls, except for s-IL-1β in severe COVID-19 patients. Among the dual role cytokines, s-IL-10 was significantly elevated in all patient groups compared to controls, whereas s-IFN-γ and s-GM-CSF were only significantly elevated in severe and/or critical COVID-19 patients. Moreover, patients with critical COVID-19 on VV-ECMO showed significantly higher leukocyte and neutrophil counts when compared to severe COVID-19 patients ($P < 0.001$), as well as higher eosinophil count when compared to severe and critical COVID-19 patients ($P < 0.05$ and $P < 0.001$, respectively). Critical COVID-19 patients presented increased neutrophil count compared to severe patients ($P < 0.01$). No differences were observed for monocyte and lymphocyte counts, but NLR was significantly higher in critical COVID-19 patients with and without VV-ECMO when compared to severe COVID-19 patients ($P < 0.05$ and $P < 0.001$, respectively) and NMR was significantly higher in patients with critical COVID-19 compared to severe patients ($P < 0.01$).

Regarding SPMs and their receptors at admission, s-RvD1 was not different between patients and controls, but s-RvE1 was significantly increased in all groups of COVID-19 patients compared to controls ($P < 0.001$), and was especially higher in patients with critical COVID-19 on VV-ECMO ($P < 0.05$ vs severe COVID-19). Conversely, the mRNA levels of $Chemerin_1$ (s-RvE1 receptor) were downregulated in all COVID-19 patients as compared to controls ($P < 0.05$ for severe COVID-19 and $P < 0.001$ for both critical groups), and were even lower in patients with COVID-19 on VV-ECMO ($P < 0.01$ vs severe COVID-19). FPR2 mRNA levels were elevated in all patient groups compared to controls ($P < 0.05$ for severe COVID-19 and $P < 0.01$ for critical groups).

3.3. Groups' differentiation by inflammatory and resolution markers – potential of RvE1-$Chemerin_1$ axis

In multivariate analysis, the PCA and PLS-DA models that best differentiated the groups were those including not only systemic cytokines (s-TNF-α, s-IL-1β, s-IL-6, s-IL-10, s-IFN-γ and s-GM-CSF), but also the resolution markers (s-RvE1, s-RvD1, FPR2 and $Chemerin_1$).

3.3.1. Healthy controls and hospitalized COVID-19 patients

We began by conducting PCA to assess the ability of inflammatory

Table 2
Inflammatory and resolutive profiles at admission of the study population.

Inflammatory and proresolving parameters	Controls (n = 23)	Severe COVID-19 (n = 27)	Critical COVID-19 (n = 17)	Critical COVID-19 on VV-ECMO (n = 17)	*P* value
Proinflammatory cytokines					
s-TNF-α (pg/mL)	11.4 (7.2; 15.1)	19.9 (13.2; 31.5)**	26.3 (18.8; 37.3)***	21.8 (14.3; 30.0)**	<0.001
s-IL-1β (pg/mL)	0.3 (0.0; 0.7)	0.9 (0.3; 1.5)	1.7 (1.2; 2.5)**	1.3 (0.8; 2.7)**	0.001
s-IL-6 (pg/mL)	0.0 (0.0; 2.9)	8.4 (5.2; 19.0)***	15.4 (5.7; 51.9)***	27.4 (4.1; 142.7)***	<0.001
Dual role cytokines					
s-IL-10 (pg/mL)	8.5 (4.2; 14.3)	37.5 (18.8; 76.5)***	50.6 (31.9; 85.2)***	31.8 (21.5; 67.3)***	<0.001
s-IFN-γ (pg/mL)	9.6 (5.7; 25.8)	32.6 (16.9; 54.8)**	32.2 (22.6; 40.8)*	22.4 (19.0; 41.3)	0.002
s-GM-CSF (pg/mL)	14.7 (4.6; 27.8)	25.6 (12.1; 45.6)	32.1 (23.6; 52.3)**	27.4 (19.8; 46.5)	0.011
Inflammatory cells					
Leukocytes (× 10^9/L)	n.d.	6 (5; 11)	9 (6; 12)	12 (9; 13)$^{\#\#\#}$	<0.001
Neutrophils (× 10^9/L)	n.d.	5 (3; 9)	9 (6; 12)$^{\#\#}$	10 (8; 12)$^{\#\#\#}$	<0.001
Eosinophils (× 10^9/L)	n.d.	0.00 (0.00; 0.01)	0.00 (0.00; 0.00)	0.02 (0.00; 0.11)$^{\#,\$\$\$}$	<0.001
Monocytes (× 10^9/L)	n.d.	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.457
Lymphocytes (× 10^9/L)	n.d.	1.0 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	0.462
NLR	n.d.	5 (3; 8)	12 (8; 17)$^{\#\#\#}$	10 (7; 13)$^{\#}$	<0.001
NMR	n.d.	11 (8; 19)	20 (15; 42)$^{\#\#}$	16 (14; 36)	0.005
Proresolving Parameters					
SPMs					
s-RvE1 (pg/mL)	416 ± 58	989 ± 81***	1247 ± 116***	1311 ± 76***, $^{\#}$	<0.001
s-RvD1 (pg/mL)	89.7 (79.3; 98.2)	90.9 (80.3; 111.2)	79.9 (68.4; 97.2)	82.2 (66.8; 101.3)	0.134
SPMs' receptors					
Chemerin$_1$ (mRNA expression)	7.0 (3.5; 11.7)	2.5 (1.1; 3.4)*	0.7 (0.3; 1.5)***	0.5 (0.1; 0.8)***, $^{\#\#}$	<0.001
FPR2 (mRNA expression)	0.2 (0.1; 0.5)	0.6 (0.4; 0.9)*	0.9 (0.4; 1.1)**	0.7 (0.5; 1.1)**	0.002

Chemerin$_1$, chemerin receptor 1; NLR, neutrophil-to-lymphocyte ratio; NMR, neutrophil-to-monocyte ratio; s-GM-CSF, serum granulocyte-macrophage colony-stimulating factor; s-IFN-γ, serum interferon gamma; s-IL-1β, serum interleukin 1 beta; s-IL-6, serum interleukin 6; s-IL-10, serum interleukin 10; s-RvD1, serum resolvin D1; s-RvE1, serum resolvin E1; s-TNF-α, serum tumor necrosis factor alpha; SPMs, specialized proresolving mediators; VV-ECMO, veno-venous extracorporeal membrane oxygenation. Results are expressed as mean ± SEM or as median (25th percentile; 75th percentile) for data with normal or non-normal distribution, respectively. *$P < 0.05$ vs Controls; **$P < 0.01$ vs Controls; ***$P < 0.001$ vs Controls; $^{\#}P < 0.05$ vs Severe; $^{\#\#}P < 0.01$ vs Severe; $^{\#\#\#}P < 0.001$ vs Severe; $^{\$\$\$}P < 0.001$ vs Critical.

and resolution parameters to distinguish between healthy controls and all COVID-19 patients or healthy controls and severe patients, as well as healthy controls and all critical patients (i.e. critical patients with and without VV-ECMO). The respective score plots are shown in Fig. 1A, Fig. 1C and Fig. 1E and demonstrate a clear separation between healthy controls and hospitalized COVID-19 patients, confirming the differences in inflammatory and resolutive profiles observed between these groups shown in Table 2. PLS-DA models were further built to identify the most relevant parameters contributing to the observed separations. We obtained significant models that differentiated healthy controls from all COVID-19 patients (R2(X) = 0.845, R2(Y) = 0.487, Q2 = 0.398, $P = 4.19 \times 10^{-8}$), healthy controls from severe patients (R2(X) = 0.854, R2(Y) = 0.576, Q2 = 0.504, $P = 1.35 \times 10^{-6}$) and healthy controls from all critical patients (R2(X) = 0.863, R2(Y) = 0.645, Q2 = 0.568, $P = 2.54 \times 10^{-8}$). VIP scores and p(corr) of all quantified variables are depicted in Fig. 1B, Fig. 1D and Fig. 1F, corresponding to the model represented above each of them. As demonstrated by these plots, s-RvE1 was the most important variable in differentiating healthy controls and hospitalized COVID-19 patients, with a VIP score > 2.5 and a p(corr) > 0.8. Notably, Chemerin$_1$ exhibited a negative p(corr) value, consistent with its higher concentrations in the control group, as opposed to s-RvE1 higher levels in COVID-19 patients (Table 2).

3.3.2. Severe and all critical COVID-19 patients

Using the 10 variables measured during the first week of hospitalization, we aimed at discriminating severe COVID-19 patients from all critical patients by PCA (Fig. 2A). This analysis did not yield a clear identification of two distinct clusters. On the other hand, PLS-DA produced a statistically significant predictive model (R2(X) = 0.732, R2(Y) = 0.197, Q2 = 0.14, $P = 6.96 \times 10^{-5}$). s-RvE1 stood out again as the most relevant parameter in differentiating these groups, with a VIP score > 2.5 and a p(corr) > 0.8 (Fig. 2B).

We then conducted univariate analyses comparing s-RvE1 and Chemerin$_1$ between the three patient groups in the first week of hospitalization. We observed that critical COVID-19 on VV-ECMO group presented markedly higher concentration of s-RvE1 than the severe group at all time points ($P < 0.05$), as well as higher levels than critical COVID-19 patients in days 3–4 and 5–8 ($P < 0.05$) (Fig. 2C). Only critical patients showed a significant reduction of s-RvE1 at days 3–4 ($P < 0.05$ vs Admission) (Fig. 2C). On the other hand, Chemerin$_1$ gene expression was significantly downregulated in all critical COVID-19 patients, especially in those on VV-ECMO, compared to severe patients at all timepoints of the first week of hospitalization (Fig. 2D). Finally, a significant reduction in Chemerin$_1$ expression was observed only in severe COVID-19 patients on days 3–4 and 5–8 ($P < 0.05$ vs Admission) (Fig. 2D).

3.3.3. COVID-19 patients requiring or not mechanical ventilation

Considering the concentrations of all inflammatory and resolution parameters during the first week of hospitalization, we performed PCA to assess the discrimination of patients requiring or not mechanical ventilation (Fig. 3A). Applying PLS-DA allowed us to create a significant model (R2(X) = 0.736, R2(Y) = 0.198, Q2 = 0.119, $P = 0.0004$), in which the distinction of mechanically ventilated patients from those not mechanically ventilated had the main contribution from s-RvE1 concentrations, as determined by a VIP score > 2.5 and a p(corr) > 0.8 (Fig. 3B).

We also performed univariate analyses to compare s-RvE1 and Chemerin$_1$ between mechanically ventilated patients and patients without mechanical ventilation. The concentration of s-RvE1 tended to be higher in mechanically ventilated patients compared to patients not requiring mechanical ventilation since admission, with this difference reaching statistical significance at days 5–8 of the first week of hospitalization ($P < 0.05$, Fig. 3C). On the other hand, Chemerin$_1$ was consistently significantly downregulated in patients requiring mechanical ventilation throughout the first week of hospitalization ($P < 0.001$, Fig. 3D).

3.3.4. Survivor and non-survivor COVID-19 patients

Mortality at 1-year post-discharge was also assessed for all COVID-19

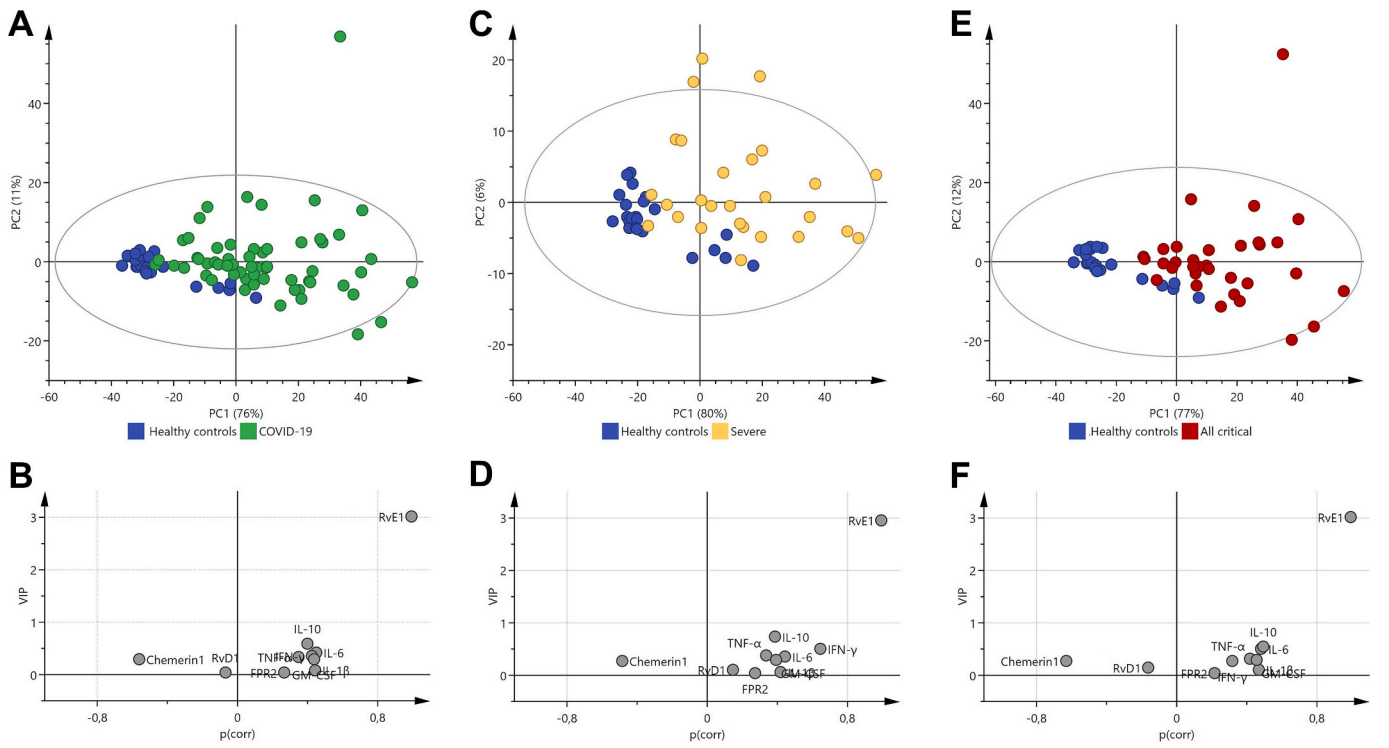

Fig. 1. Healthy controls and hospitalized COVID-19 patients' profiling by inflammatory and resolution markers at admission. (A) Score plot of PCA of 23 healthy controls and 60 COVID-19 patients ($N = 83$). The first two components explained 76 % and 11 % of the data variation, respectively. (B) VIP scores and p(corr) of all variables included in the PLS-DA model distinguishing healthy controls from all COVID-19 patients. (C) Score plot of PCA of 23 healthy controls and 27 severe COVID-19 patients ($N = 50$). The first two components explained 80 % and 6 % of the data variation, respectively. (D) VIP scores and p(corr) of all variables included in the PLS-DA model distinguishing healthy controls from severe COVID-19 patients. (E) Score plot of PCA of 23 healthy controls and 33 critical COVID-19 patients ($N = 56$). The first two components explained 77 % and 12 % of the data variation, respectively. (F) VIP scores and p(corr) of all variables included in the PLS-DA model distinguishing healthy controls from all critical COVID-19 patients. p(corr), correlation coefficient; PC, principal component; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; VIP, variable importance in projection.

patients and PCA was performed to differentiate survivors from non-survivors based on inflammatory and resolution parameters (Fig. 4A). Although the PCA did not readily discriminate between the groups, the PLS-DA enabled us to generate a model that significantly separated COVID-19 survivors from non-survivors (R2(X) = 0.734, R2(Y) = 0.25, Q2 = 0.202, $P = 3.60 \times 10^{-7}$), primarily driven by the contribution of s-RvE1 (VIP score > 2.5 and p(corr) > 0.8) (Fig. 4B).

Additionally, we conducted univariate analyses that confirmed the significant difference between 1-year survivor and non-survivor COVID-19 patients regarding s-RvE1 concentrations. Indeed, s-RvE1 was significantly increased in COVID-19 non-survivors at days 3–4 and 5–8 of the first week of hospitalization (Fig. 4C). Conversely, its receptor, $Chemerin_1$, tended to be downregulated in non-survivors during the two first time points of the first week of hospitalization, and significantly so at days 5–8 ($P < 0.01$).

3.4. Correlation analysis at admission in all patients

Next, we evaluated the correlations for s-RvE1 and $Chemerin_1$ with inflammatory and respiratory parameters and with hospital length of stay, for all patients at admission. We observed that s-RvE1 was associated with inflammatory status, presenting significant positive correlations with proinflammatory and dual role cytokines, such as s-IL-1β, s-IL-6, s-IFN-γ and s-GM-CSF (Fig. 5A-D), as well as with inflammatory cells, namely leucocyte count, neutrophil count, monocyte count and NLR (Fig. 5E-H). Moreover, s-RvE1 was also positively correlated with $PaCO_2$ (Fig. 5I).

On the other hand, we observed significant inverse correlations of $Chemerin_1$ with inflammatory parameters, such as leucocyte count, neutrophil count, NLR and NMR in all patients at admission (Fig. 6A-D). $Chemerin_1$ was also inversely correlated with total hospital length of stay and $PaCO_2$, but positively correlated with the PaO_2/FiO_2 ratio (Fig. 6E-G).

3.5. Repeated measures multivariate analyses

When considering s-RvE1 as the dependent variable, we observed a significant positive association with critical on VV-ECMO group (*Model 1*: Adjusted β: 370.57; $P < 0.001$) and a significant inverse association with survival within 1-year post-hospital discharge (*Model 3*: Adjusted β: −307.80; $P = 0.007$) (Table 3). Additionally, a borderline inverse association was found between s-RvE1 and the absence of mechanical ventilation (*Model 2*: Adjusted β: −155.72; $P = 0.081$) (Table 3).

Regarding $Chemerin_1$ associations with the same independent variables, we observed that both critical groups were associated with significantly lower $Chemerin_1$ mRNA expression (*Model 4*: Critical – Adjusted β: −1.15; $P = 0.002$; Critical on VV-ECMO – Adjusted β: −1.51; $P < 0.001$), while survival was associated with significantly higher $Chemerin_1$ mRNA expression (*Model 6*: Adjusted β: 0.59; $P = 0.013$) (Table 3). There was also a borderline positive association between $Chemerin_1$ and the absence of mechanical ventilation (*Model 5*: Adjusted β: 0.65; $P = 0.056$) (Table 3). Moreover, only for this model, female sex appeared to be an independent predictor of lower $Chemerin_1$ mRNA expression (*Model 5*: Adjusted β: −0.49; $P = 0.029$).

4. Discussion

The present study highlights a prominent role for RvE1-$Chemerin_1$ axis in the pathophysiology of COVID-19. Here, we reveal that, among a panel including several systemic cytokines, resolvins and their

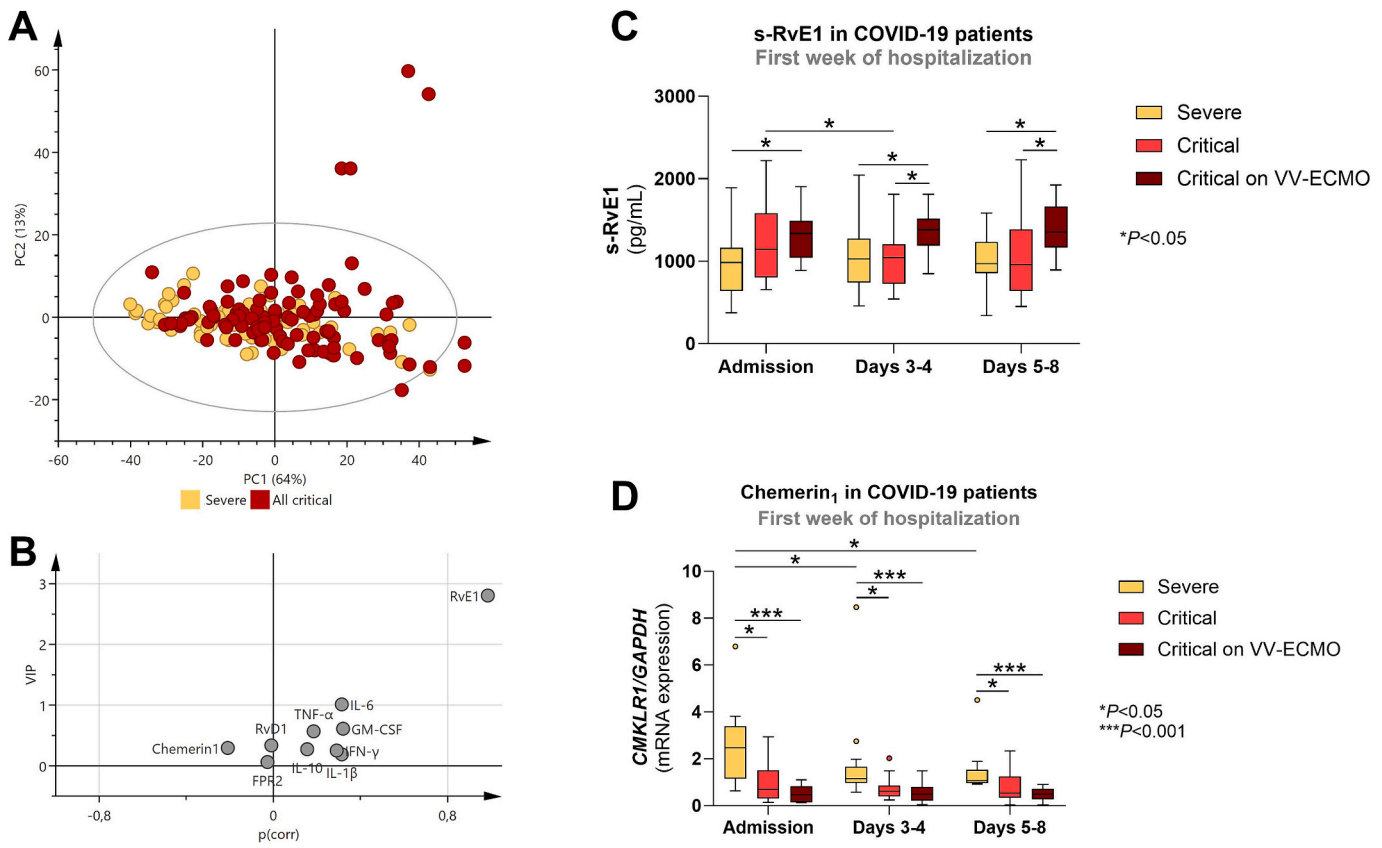

Fig. 2. Severe and all critical COVID-19 patients' profiling by inflammatory and resolution markers during the first week of hospitalization. (A) Score plot of PCA of 27 severe COVID-19 patients and 33 critical COVID-19 patients ($N = 160$ for overall observations for the three time points). The first two components explained 64 % and 13 % of the data variation, respectively. (B) VIP scores and p(corr) of all variables included in the PLS-DA model distinguishing severe from all critical COVID-19 patients. (C) s-RvE1 concentration in COVID-19 patient groups during the first week of hospitalization ($N = 27$, 19 and 16, for severe patients at admission, days 3–4 and days 5–8, respectively; $N = 17$, 17 and 14, for critical patients at admission, days 3–4 and days 5–8, respectively; N = 17, 17 and 17, for critical patients on VV-ECMO at admission, days 3–4 and days 5–8, respectively). (D) $Chemerin_1$ (*CMKRL1*) expression in COVID-19 patient groups during the first week of hospitalization ($N = 18$, 15 and 11, for severe patients at admission, days 3–4 and days 5–8, respectively; $N = 15$, 13 and 12, for critical patients at admission, days 3–4 and days 5–8, respectively; $N = 13$, 14 and 14, for critical patients on VV-ECMO at admission, days 3–4 and days 5–8, respectively). $Chemerin_1$, chemerin receptor 1; *CMKRL1*, $Chemerin_1$ gene; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase gene; p(corr), correlation coefficient; PC, principal component; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; s-RvE1, serum resolvin E1; VIP, variable importance in projection; VV-ECMO, veno-venous extracorporeal membrane oxygenation.

receptors, RvE1 was the mediator that best distinguished hospitalized COVID-19 patients from healthy controls or patients with different disease severity, also discriminating between patients requiring or not mechanical ventilation and between non-survivors from survivors. Accordingly, RvE1 was increased in all COVID-19 patients compared to healthy controls and, within patients, it was significantly higher in those under VV-ECMO, in patients requiring mechanical ventilation and in non-survivors. In contrast, its receptor $Chemerin_1$ exhibited the opposite profile in all these patients. Moreover, RvE1 and $Chemerin_1$ presented positive and inverse correlations, respectively, with inflammation, respiratory function and hospital length of stay. These results further emphasize the dysregulated resolution response in hospitalized COVID-19 patients, particularly in those with critical illness.

Resolution of inflammation pathways have been scarcely explored in COVID-19. Moreover, the results obtained in the few existing studies appear to be contradictory. Some studies showed reduced amounts of SPMs and their biosynthetic intermediates in the peripheral blood of critical COVID-19 patients compared to those with severe disease [23,26,67], while others referred raised levels of SPMs in the serum and lungs of patients with critical disease compared to severe patients or healthy controls [21,22,24,61]. Alongside the evaluation of SPMs, most of these studies and others have also focused on the assessment of eicosanoids, reporting increased levels of prostaglandins, thromboxanes and leukotrienes in blood, tracheal aspirates and bronchoalveolar lavages of COVID-19 patients [21,61,68]. Accordingly, both their precursor, arachidonic acid, and the enzyme responsible for its biosynthesis, phospholipase A_2, have been found to be elevated in COVID-19 patients and correlated with disease severity [69]. Moreover, in a study conducted by our group on the same cohort of COVID-19 patients, we investigated the relationship of cysteinyl leukotrienes with disease severity. We observed that although urinary cysteinyl leukotrienes did not differ between groups at admission, they significantly increased along hospitalization only in critical groups, being markedly higher in VV-ECMO patients, especially in hypertensives [65]. This study also highlighted the prognostic potential of urinary cysteinyl leukotrienes values during the first week of hospitalization, which were associated with severe outcomes and demonstrated strong predictive value for 30-day mortality [65]. However, some studies have reported lower eicosanoids levels in critically ill patients compared to those with severe disease, including the works of Palmas et al. and Biagini et al. that reported the same pattern for SPMs [26,28,67]. In our present study, we detected higher concentrations of RvE1 in all COVID-19 patients at admission, with increasing values alongside disease severity. On the other hand, RvD1 concentrations did not differ between groups. Our group has also previously shown distinct profiles of RvE1 and RvD1 in other critical care patients, with RvE1, but not RvD1, increasing with

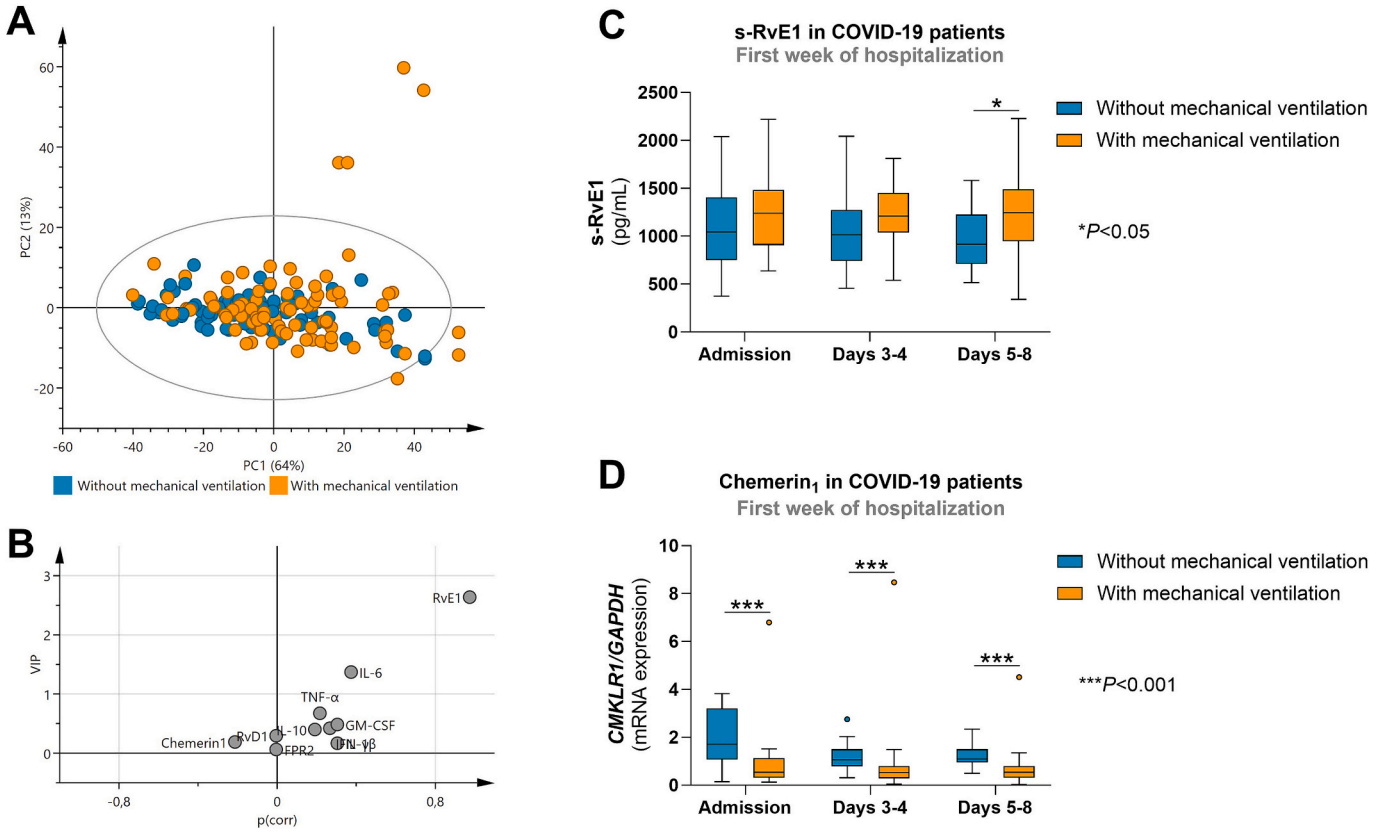

Fig. 3. COVID-19 patients requiring or not mechanical ventilation – profiling by inflammatory and resolution markers during the first week of hospitalization. (A) Score plot of PCA of 29 mechanically ventilated COVID-19 patients and 31 COVID-19 patients not mechanically ventilated (N = 160 for overall observations for the three time points). The first two components explained 64 % and 13 % of the data variation, respectively. (B) VIP scores and p(corr) of all variables included in the PLS-DA model distinguishing COVID-19 patients requiring or not mechanical ventilation. (C) s-RvE1 concentration in mechanically ventilated vs not mechanically ventilated COVID-19 patients at admission (*N* = 30 vs 31), days 3–4 (N = 30 vs 23) and days 5–8 (N = 30 vs 17). (D) Chemerin$_1$ (*CMKRL1*) expression in mechanically ventilated vs not mechanically ventilated COVID-19 patients at admission (*N* = 24 vs 22), days 3–4 (*N* = 22 vs 20) and days 5–8 (*N* = 25 vs 12). Chemerin$_1$, chemerin receptor 1; *CMKRL1*, Chemerin$_1$ gene; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase gene; p(corr), correlation coefficient; PC, principal component; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; s-RvE1, serum resolvin E1; VIP, variable importance in projection.

disease severity [70]. Furthermore, in a preliminary ex vivo experiment conducted at our lab, where we incubated whole blood samples from two patients (one with acute heart failure and one with cardiogenic shock) and two healthy controls with the calcium ionophore A23187 (50 μM) and/or the omega-3 PUFAs DHA (1 μg/mL) and EPA (1 μg/mL), we also noted a marked increase in RvE1 levels following the addition of the calcium ionophore A23187, whereas RvD1 concentrations remained similar (supplementary data, Fig. S2). These consistent findings suggest potential differences in the activation of RvD1 pathways compared to RvE1. The biosynthesis of RvE1 first depends on cyclooxygenase (COX)-2 or cytochrome P450 (CYP450) and then on 5-lipoxygenase (5-LOX) [43], whereas the biosynthesis of RvD1 involves the action of 15-lipoxygenase (15-LOX) followed by 5-LOX [71]. Thus, the enhancement of biosynthetic pathways involving CYP450 and 5-LOX might explain the significant rise of RvE1 in highly inflammatory settings. Accordingly, Schwarz et al. reported an increase of 5-LOX and CYP450-derived lipid mediators in COVID-19 patients with higher severity [28]. Also, in a previous study on mice infected with two strains of influenza, 5-LOX and CYP450-derived mediators were strongly correlated with the more pathogenic strain [72].

Importantly, we show that RvE1 was the mediator best discriminating between healthy controls and COVID-19 patients, as well as between patients with severe and critical disease, consistently achieving VIP scores greater than 2.5. These results are in accordance with findings by Irún et al. who, through a lipidomic approach using liquid chromatography tandem mass spectrometry (LC-MS/MS), also demonstrated an increase of RvE1 in the serum of critical COVID-19 patients and highlighted its significant contribution (VIP > 1.5) in distinguishing between controls and COVID-19 patients [22]. Still in agreement with our results, RvD1 concentrations did not differ between their study groups [22]. Despite many other researchers not mentioning or detecting RvE1, the bioactive intermediate of its biosynthesis, 18-hydroxyeicosapentaenoic acid (18-HEPE), was also often found to be elevated in COVID-19 patients compared to controls [21,24,28,61]. Noteworthy, 18-HEPE was previously proposed as a biomarker for monitoring the activation of the EPA-RvE1-Chemerin$_1$ axis towards inflammation resolution [73]. This particular activation of the RvE1 pathway suggests its potential role in COVID-19 disease or possibly in infections in general, considering the reported benefits of RvE1 administration in mice with *Escherichia coli* pneumonia or ocular herpes simplex virus [53,54,74]. Other studies have also reported a protective effect of RvE1 treatment on bacterial growth or microbiota profile [75,76]. Moreover, RvE1 presented positive correlations with both proinflammatory (IL-1β and IL-6) and dual role cytokines (IFN-γ and GM-CSF), but not with IL-10. In fact, while both GM-CSF and IL-10 have been suggested to exert protective effects in the early stages of infection, their sustained elevation at later stages may contribute to systemic inflammation [77,78]. Thus, in our study, the increase of RvE1 with disease severity and its positive correlation with inflammation could indicate an effort to counteract the infection and the proinflammatory state. In fact, critical COVID-19 patients exhibited a higher use of antibiotics due to bacterial co-infections, which might have been an additional stimulus for RvE1 production. It could

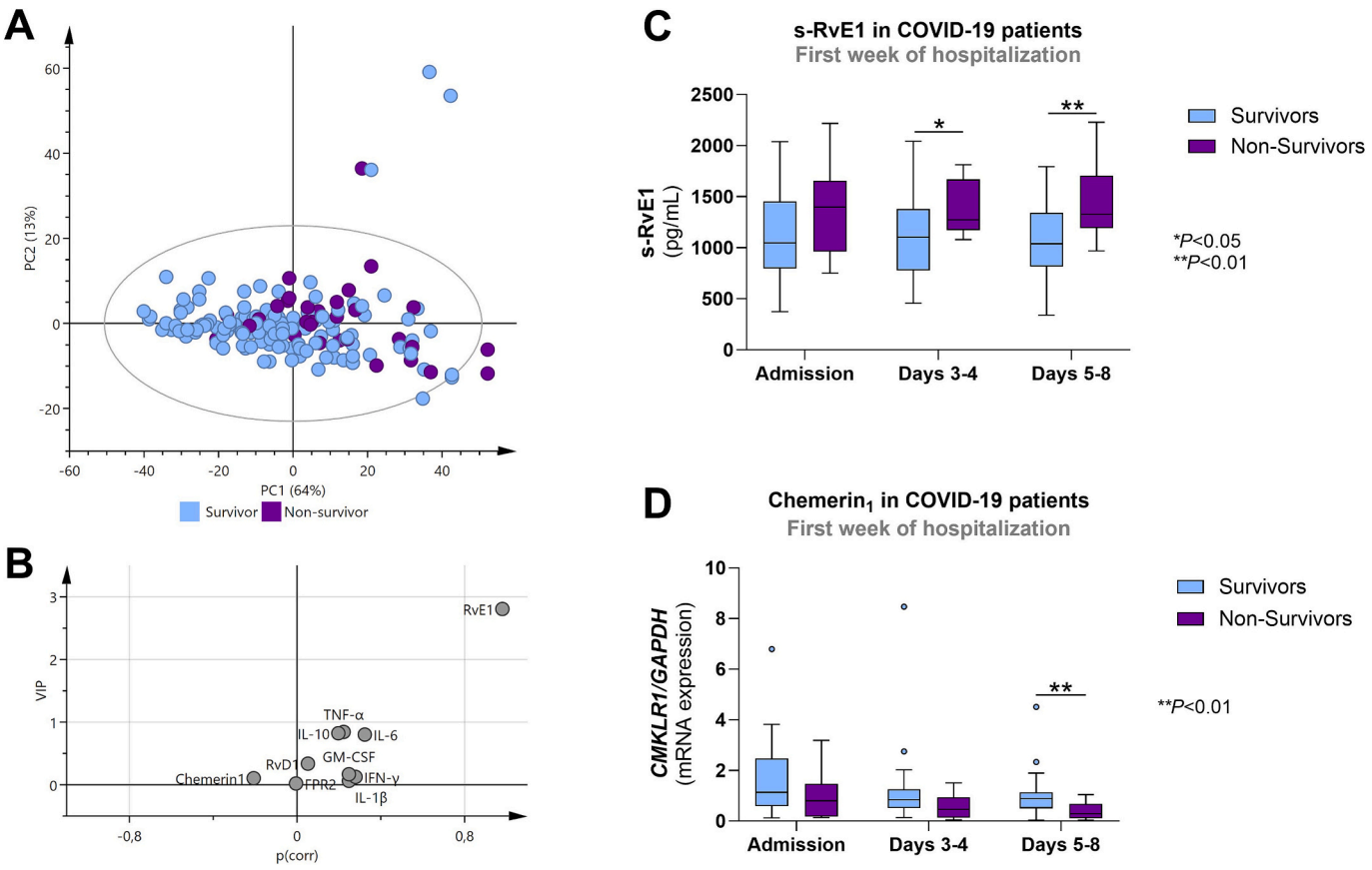

Fig. 4. COVID-19 patients 1-year survivors and non-survivors – profiling by inflammatory and resolution markers during the first week of hospitalization. (A) Score plot of PCA of 12 COVID-19 survivors and 47 non-survivors ($N = 157$ for overall observations for the three time points). The first two components explained 64 % and 13 % of the data variation, respectively. (B) VIP scores and p(corr) of all variables included in the PLS-DA model distinguishing COVID-19 patients 1-year survivors from non-survivors. (C) s-RvE1 concentration in survivors vs non-survivors COVID-19 patients at admission ($N = 48$ vs 12), days 3–4 ($N = 43$ vs 9) and days 5–8 ($N = 36$ vs 10). (D) Chemerin$_1$ (*CMKRL1*) expression in survivors vs non-survivors COVID-19 patients at admission ($N = 37$ vs 8), days 3–4 ($N = 35$ vs 6) and days 5–8 ($N = 31$ vs 5). Chemerin$_1$, chemerin receptor 1; *CMKRL1*, Chemerin$_1$ gene; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase gene; p(corr), correlation coefficient; PC, principal component; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; s-RvE1, serum resolvin E1; VIP, variable importance in projection.

also be speculated that the administered therapeutics might have interfered with RvE1 concentrations. While dexamethasone has been reported to promote SPM production in experimental allergic inflammation [79] and in COVID-19 patients [23], this effect was not observed for RvE1. Additionally, a recent study by Rao et al. suggests that glucocorticoids influence SPM levels via 15-LOX [80]. However, few studies have examined this mechanism or the interplay between pro-resolving mediators, making it difficult to rule out an indirect effect of glucocorticoids on RvE1. It is worth noting that in our cohort dexamethasone was administered to nearly all patients, thus influencing all groups similarly. On the other hand, in the study by Irún et al., less than 50 % of patients in each group received glucocorticoids [22]. While both studies reported increased RvE1 concentrations in critical COVID-19 patients, we additionally observed a significant elevation in severe patients, which suggests that dexamethasone may contribute more prominently to the rise in RvE1 in severe cases than in critical ones. Regrettably, since all blood samples were collected after treatment initiation, we were unable to evaluate the specific impact of dexamethasone or antibiotics. Beyond these therapeutics, vaccination against SARS-CoV-2 has also been shown to influence the inflammatory response during COVID-19 [81], although its impact on proresolving mediators remains unknown. This could not be addressed in our study, as our cohort included only non-immunized COVID-19 patients.

To further investigate resolvins' network in COVID-19 patients, we assessed the gene expression of their receptors on PBMCs. Interestingly, we observed a downregulation of the RvE1 receptor, Chemerin$_1$, closely mirroring the upregulation of RvE1 across our study groups. Very few studies have evaluated the expression levels of the RvE1 receptor in COVID-19 patients, reporting reduced expression in classical and intermediate monocytes of more severe patients, as well as in natural killer (NK) cells of non-hospitalized, hospitalized and ICU patients compared to healthy controls [23,82]. Indeed, human Chemerin$_1$ gene – *CMKLR1* – is mainly expressed in dendritic cells, monocytes and macrophages, as well as in NK cells [83,84], and the in vitro activation of these cells by LPS or cytokines has been shown to downregulate its expression [85,86]. In the present study, we observed significant inverse correlations between Chemerin$_1$ and inflammatory cells, which corroborate the hypothesis of a reduction in receptor expression in response to a hyperstimulating inflammatory state. This inverse relationship has also been confirmed in Chemerin$_1$ knockout mice [87]. Therefore, the increasing concentration of RvE1 in COVID-19 patients does not appear to be accompanied by the proresolutive effects reported when interacting with Chemerin$_1$ [43]. Interestingly, chemerin, the first known endogenous agonist of Chemerin$_1$, has also been found to be increased in COVID-19 patients compared to healthy controls, as well as in patients that did not survive compared to recovered ones [82]. Given that RvE1 and chemerin activate different Chemerin$_1$ downstream signaling pathways [83], it would be relevant to further differentiate the actions of each agonist during infection. Nonetheless, RvE1 also appears to signal through the BLT$_1$ receptor [29,48,53], which neither we nor other

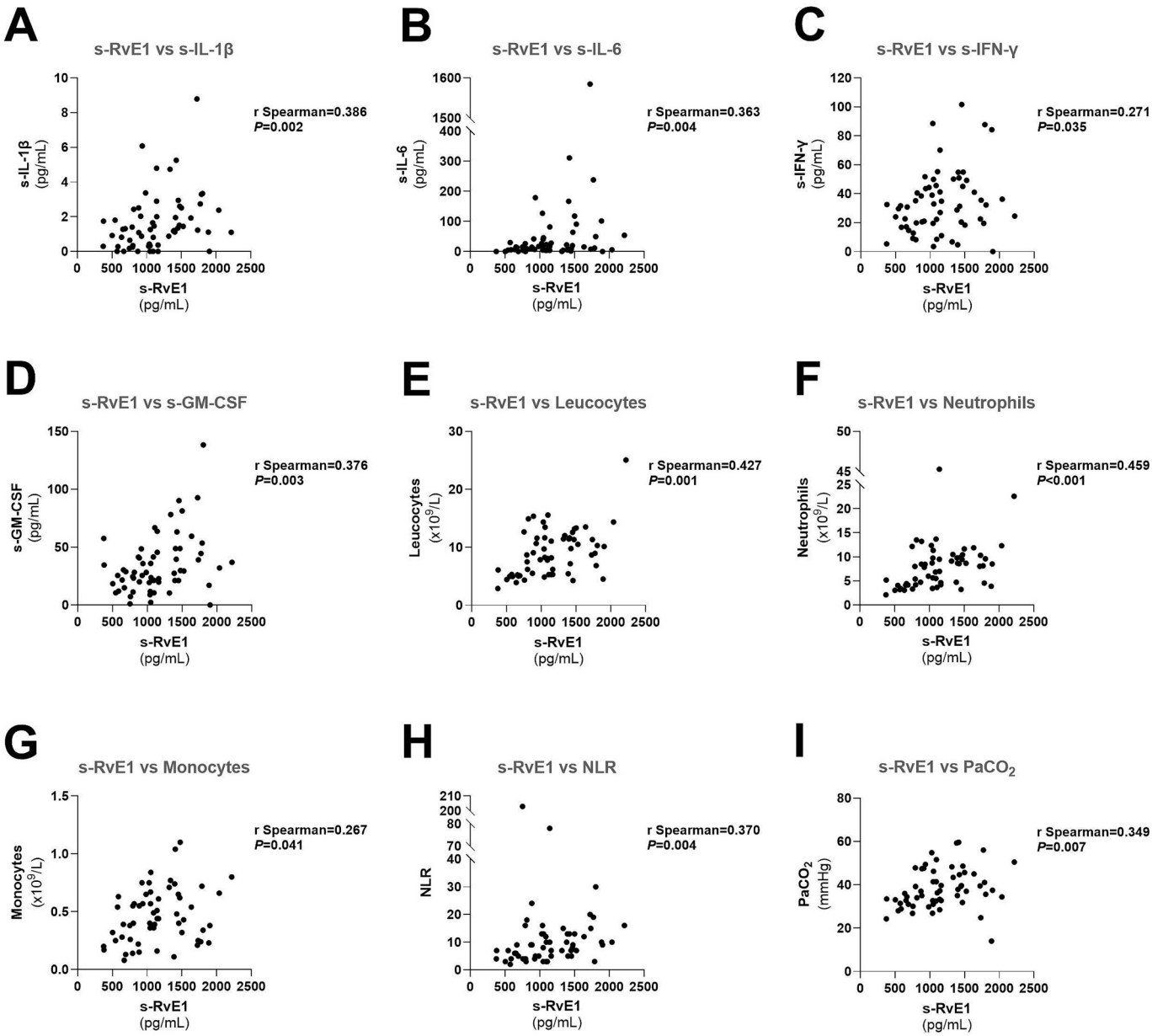

Fig. 5. Spearman correlations for s-RvE1 in all patients at admission: (A) s-RvE1 vs s-IL-1β; (B) s-RvE1 vs s-IL-6; (C) s-RvE1 vs s-IFN-γ; (D) s-RvE1 vs s-GM-CSF; (E) s-RvE1 vs Leucocytes; (F) s-RvE1 vs Neutrophils; (G) s-RvE1 vs Monocytes; (H) s-RvE1 vs NLR; (I) s-RvE1 vs $PaCO_2$. NLR, neutrophil-to-lymphocyte ratio; $PaCO_2$, partial pressure of carbon dioxide; s-GM-CSF, serum granulocyte-macrophage colony-stimulating factor; s-IFN-γ, serum interferon gamma; s-IL-1β, serum interleukin 1-beta; s-IL-6, serum interleukin 6; s-RvE1, serum resolvin E1.

researchers have studied in COVID-19 patients. However, its primary endogenous agonist, leukotriene B_4, has been found at elevated levels in more severe COVID-19 cases [23]. Therefore, we cannot rule out the possibility that BLT_1 contributes to RvE1 proresolving effects in these patients.

In our patient cohort, the RvE1-$Chemerin_1$ axis was also associated with disease severity and mortality. In fact, in univariate analyses critical COVID-19 patients on VV-ECMO showed significantly higher RvE1 and lower $Chemerin_1$ levels compared to severe patients, from admission until the end of the first week of hospitalization. Likewise, mechanically ventilated patients and non-survivors exhibited the same profile compared to patients not requiring mechanical ventilation and survivors, respectively. Repeated measures multivariate analyses reinforced the association between higher disease severity and worse prognosis with elevated RvE1 levels and reduced $Chemerin_1$ mRNA expression. Through PLS-DA, RvE1 emerged once again as the most significant parameter in distinguishing these patients. Accordingly, Dalli et al. found higher amounts of RvE1 in sepsis non-survivors compared to surviving subjects [60]. Also, in a mice model of viral pneumonia, $Chemerin_1$-deficient mice had delayed clearance of the virus and higher mortality [59]. These results, together with the positive correlation of RvE1 with $PaCO_2$, and the inverse correlations of $Chemerin_1$ with respiratory function and hospitalization length of stay, highlight the protective role of this receptor in pulmonary infections and its dysregulation in hospitalized COVID-19 patients.

Notably, no significant sex differences were observed across our study groups, nor between mechanically ventilated and non-mechanically ventilated patients or survivors and non-survivors (supplementary data, Table S2). Consistently, the results of multivariate analyses adjusted for sex aligned with univariate analysis findings, indicating no major impact of sex on clinical outcomes, except in the model evaluating the association of $Chemerin_1$ with the need for

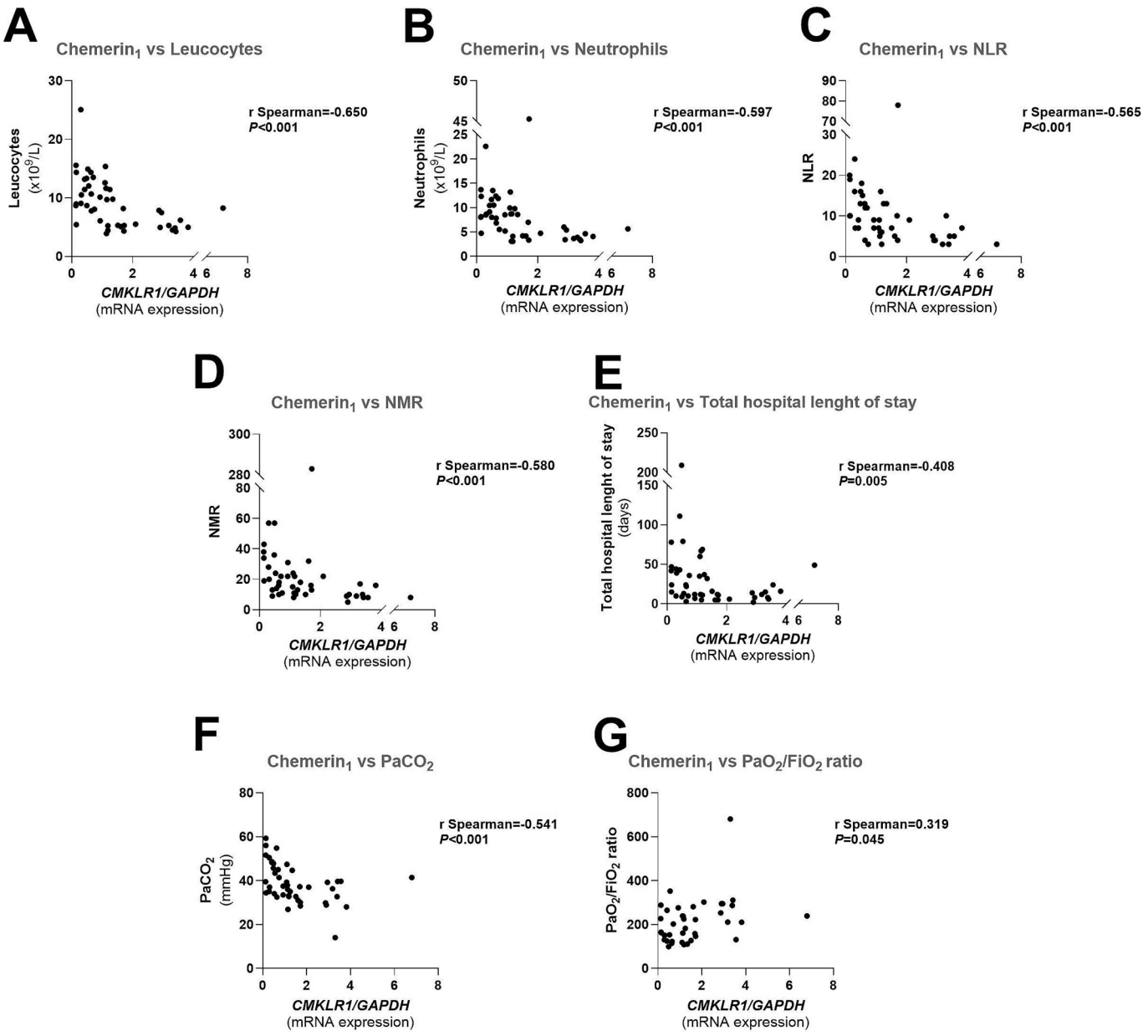

Fig. 6. Spearman correlations for Chemerin$_1$ (*CMKRL1*) expression in all patients at admission: (A) Chemerin$_1$ vs Leucocytes; (B) Chemerin$_1$ vs Neutrophils; (C) Chemerin$_1$ vs NLR; (D) Chemerin$_1$ vs NMR; (E) Chemerin$_1$ vs Total hospital length of stay; (F) Chemerin$_1$ vs PaCO2; (G) Chemerin$_1$ vs PaO_2/FiO_2 ratio. Chemerin$_1$, chemerin receptor 1; NLR, neutrophil-to-lymphocyte ratio; NMR, neutrophil-to-monocyte ratio; $PaCO_2$, partial pressure of carbon dioxide; PaO_2/FiO_2 ratio, ratio of partial pressure of oxygen and fraction of inspired oxygen.

mechanical ventilation, where female sex appeared to be an independent predictor of lower Chemerin$_1$ mRNA expression.

Regarding the RvD1-FPR2 axis, the upregulation of FPR2 receptor in all of our COVID-19 patients might have been induced by dexamethasone, since this treatment has been shown to stimulate FPR2 expression in leukocytes [88,89]. In contrast to our results, a previous study in COVID-19 patients found no differences in FPR2 expression in neutrophils and leukocytes between healthy volunteers and COVID-19 patients, nor between patients with different disease severity, and further showed that FPR2 expression was downregulated in patients treated with dexamethasone [23]. Since FPR2 is also a target for other ligands, including proresolving (e.g. lipoxin A_4, annexin A1) and proinflammatory molecules (e.g. serum amyloid A, amyloid beta 42) [90], we cannot exclude that changes in the concentrations of these mediators might have influenced FPR2 expression in our patients. Furthermore, proinflammatory cytokines such as TNF-α may also upregulate FPR2 expression [91]. Indeed, we also observed that FPR2 was positively correlated with TNF-α concentrations within patients (data not shown).

Our findings further suggest that dysregulated proresolving pathways may contribute to sustained hyperinflammatory responses, exacerbating disease severity. Notably, despite the increase in RvE1, likely as a compensatory response to mitigate hyperinflammation, and the use of dexamethasone, Chemerin$_1$ expression remained low, probably impairing resolution through this axis. Thus, novel therapeutic strategies aimed at stimulating endogenous resolution pathways could complement the anti-inflammatory effects of existing treatments, such as glucocorticoids, and help prevent critical clinical conditions [92]. In hospitalized COVID-19 patients, targeting the receptor Chemerin$_1$ to restore its expression may enhance the protective bioactions of RvE1. This approach has been tested in macrophages with reduced *GPR32* expression following stimulation with the SARS-CoV-2 spike 1 glycoprotein, where treatment with exogenous RvD1 reversed the suppressive

Table 3
Repeated measures multivariate models for s-RvE1 and Chemerin$_1$ in all COVID-19 patients during the first week of hospitalization.

s-RvE1 (pg/mL)	Adjusted β	95 % CI	*P* value
Model 1: COVID-19 Group			
Severe	Ref		
Critical	115.18	−97.33 to 327.68	0.288
Critical on VV-ECMO	370.57	184.70 to 556.44	<0.001[a]
Model 2: Mechanical Ventilation			
Yes	Ref		
No	−155.72	−330.74 to 19.30	0.081
Model 3: Mortality within 1-year post-hospital discharge			
Yes	Ref		
No	−307.80	−531.84 to −83.76	0.007[a]
Chemerin$_1$ (mRNA expression)	**Adjusted β**	**95 % CI**	***P* value**
Model 4: COVID-19 Group			
Severe	Ref		
Critical	−1.15	−1.87 to −0.44	0.002[a]
Critical on VV-ECMO	−1.51	−2.30 to −0.71	<0.001[a]
Model 5: Mechanical Ventilation			
Yes	Ref		
No	0.65	−0.02 to 1.32	0.056
Model 6: Mortality within 1-year post-hospital discharge			
Yes	Ref		
No	0.59	0.13 to 1.05	0.013[a]

(Adjusted β), 95 % confidence intervals (95 % CI) and *p* value estimated by repeated measures multivariate models with s-RvE1 and Chemerin$_1$ as the dependent variables and adjusted for age and sex. [a]All associations with $P < 0.05$ were considered significant.
Chemerin$_1$, chemerin receptor 1; Ref, reference; s-RvE1, serum resolvin E1; VV-ECMO, veno-venous extracorporeal membrane oxygenation.

effect [93].

4.1. Limitations of the study

Firstly, this is a study with reduced sample size and with a single-center design, which may introduce selection bias due to consecutive recruitment. In addition, we were not always able to collect blood samples at all time points of the first week of hospitalization from all ward patients. This was due to the heavy clinical workload during the COVID-19 pandemic and patient withdrawal of consent often stemming from fear and psychological distress experienced by hospitalized COVID-19 patients. Since this issue exclusively affected the severe patient group, a reduction in statistical power, particularly in analyses throughout hospitalization, cannot be ruled out, potentially impacting the results within this group. However, as shown in supplementary data (Fig. S1), the final sample sizes at days 5–8 were comparable across groups ($N = 16$ for severe; $N = 14$ for critical; $N = 17$ for critical patients on VV-ECMO).

Finally, we quantified resolvins using ELISA kits, which are not the gold standard methodology for SPMs analysis since the antibodies used may have some degree of cross-reactivity with other SPMs, their precursors and metabolites with very similar molecular structures [94]. This cross-reactivity can lead to overestimation of resolvin concentrations, as their precursors are typically more abundant, potentially affecting data accuracy. Although the manufacturer has reported lack of cross-reactivity with the analogues RvD2, RvD4 and RvD6 for the Human Resolvin D1 kit or with the analogues RvE2, RvD1, RvD2 and RvD3 for the Human Resolvin E1 kit, these studies may be insufficient, and additional testing with structurally similar molecules is warranted. However, the mean concentration of RvE1 in our healthy controls is consistent with other studies that measured RvE1 using ELISA, including those employing kits that report low cross-reactivity (<0.001 %) with the precursors EPA and 18-HEPE [46]. Accordingly, in our laboratory, the concentrations of RvD1 and RvE1 quantified by ELISA showed no changes following incubation with the precursors DHA and EPA, in preliminary ex vivo experiments using whole blood from healthy controls and patients (supplementary data, Fig. S2). Our choice of measuring resolvins by ELISA was based on the availability of equipment in our facilities, as well as on the higher feasibility for implementing these assays into routine hospital analyses compared to the LC-MS/MS methodology, which requires expensive equipment and highly specialized and trained human resources. Nevertheless, our results of increased RvE1 in critical patients are in accordance with Irún et al. findings using LC-MS/MS [22], despite the different proportion of dexamethasone treated patients in the two studies, which suggests that disease severity in critical patients may play a greater role in the increase of RvE1 than dexamethasone treatment. Moreover, the significant changes also found for RvE1 receptor expression further reinforce the relevance of this RvE1-Chemerin$_1$ axis in COVID-19 patients.

5. Conclusions

Collectively, our results demonstrate the particular importance of RvE1 in distinguishing COVID-19 disease severity over a panel including several cytokines, RvD1, RvE1 and the proresolving receptors FPR2 and Chemerin$_1$. Additionally, the upregulation of RvE1 in critical COVID-19 patients, as well as in mechanically ventilated patients and non-survivors, accompanied by the downregulation of its receptor Chemerin$_1$, suggests a failure of this proresolving axis in hospitalized COVID-19 patients. These findings highlight the pertinence of evaluating resolution pathways along with acute inflammatory profiles in all diseases that are associated with inflammation. Furthermore, given the significant correlations with several respiratory parameters observed for RvE1 and its receptor, it might be useful to explore this proresolving axis in other lung diseases.

CRediT authorship contribution statement

Carolina Silva-Pereira: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Marta Reina-Couto:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis, Conceptualization. **Patrícia Pereira-Terra:** Writing – review & editing, Investigation, Formal analysis. **Luísa Teixeira-Santos:** Writing – review & editing, Investigation, Formal analysis. **Sandra Martins:** Investigation, Formal analysis. **Dora Pinho:** Writing – review & editing, Investigation, Formal analysis. **Miguel Luz Soares:** Writing – review & editing, Investigation. **Cláudia Camila Dias:** Writing – review & editing, Formal analysis. **António Sarmento:** Writing – review & editing, Resources. **Margarida Tavares:** Writing – review & editing, Resources. **João Tiago Guimarães:** Writing – review & editing, Resources. **José-Artur Paiva:** Writing – review & editing, Resources. **Sónia Fraga:** Writing – review & editing. **António Albino-Teixeira:** Writing – review & editing, Resources, Funding acquisition. **Roberto Roncon-Albuquerque:** Writing – review & editing, Supervision, Resources, Conceptualization. **Teresa Sousa:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2025.115669.

Data availability

Data will be made available on request.

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