



OPEN sST2 is a key outcome biomarker in COVID-19: insights from discovery randomized trial

David M. Smadja^{1,2,109}✉, Clément R. Massonnaud^{3,4,109}, Aurélien Philippe^{1,2,110}, Mickael Rosa^{5,110}, Sophie Luneau², Antoine Rauch⁵, Nathan Peiffer-Smadja^{4,6}, Amandine Gagneux-Brunon^{7,8}, Julien Poissy⁵, Maxime Gruet², Alexandre Ung⁵, Valérie Pourcher⁹, François Raffi¹⁰, Lionel Piroth¹¹, Kévin Bouiller¹², Hélène Esperou¹³, Christelle Delmas⁴, Drifa Belhadi^{3,4}, Alpha Diallo¹⁴, Juliette Saillard¹⁴, Aline Dechanet^{3,4}, Noémie Mercier¹⁴, Axelle Dupont^{4,6}, François-Xavier Lescure^{4,6}, François Goehringer¹⁵, Stéphane Jaureguiberry^{16,17}, François Danion¹⁸, Violaine Tolsma¹⁹, André Cabie^{20,21}, Johan Courjon^{22,23}, Sylvie Leroy^{24,25,26}, Joy Mootien²⁷, Bruno Mourvillier²⁸, Sébastien Gallien^{29,30}, Jean-Philippe Lanoix^{31,32}, Elisabeth Botelho-nevers^{7,8}, Florent Wallet³³, Jean-Christophe Richard^{34,35}, Jean Reuter³⁶, Alexandre Gaymard^{37,38}, Richard Greil³⁹, Guillaume Martin-Blondel^{40,41}, Claire Andrejak⁴², Yazdan Yazdanpanah^{4,6}, Charles Burdet^{3,4}, Jean-Luc Diehl^{1,43}, Maya Hites⁴⁴, Florence Ader^{45,46}, Sophie Susen⁵, France Mentre^{3,4,111}, Annabelle Dupont^{5,111} & the Discovery Study Group*

We investigated whether baseline levels of biomarkers related to endotheliopathy, thromboinflammation, and fibrosis were associated with clinical outcomes in hospitalized COVID-19 patients. We analyzed the associations between baseline levels of 21 biomarkers and time to hospital discharge and change in NEWS-2 score in patients from DisCoVeRy trial. We fitted multivariate models adjusted for baseline ISARIC 4C score, disease severity, D-dimer values, and treatment regimen. Between March 22 and June 29, 2020, 603 participants were randomized; 454 had a sample collected at baseline and analyzed. The backward selection of multivariate models showed that higher baseline levels of soluble suppressor of tumorigenicity 2 (sST2) and nucleosomes were statistically associated with a lower chance of hospital discharge before day 29 (sST2: aHR 0.24, 95% CI [0.15–0.38], $p < 10^{-9}$; nucleosomes: aHR 0.62, 95% CI [0.48–0.81], $p < 10^{-3}$). Likewise, higher levels of baseline sST2 were statistically associated with lower changes in the NEWS-2 score between baseline and day 15 (adjusted beta 4.47, 95% CI [2.65–6.28], $p < 10^{-5}$). Moreover, we evaluated sST2 involvement in a confirmation cohort (SARCODO study, 103 patients) and found that elevated baseline sST2 levels were significantly associated with lower rates of hospital discharge before day 29 and a higher model performance (AUC at day 29 of 92%) compared to models without sST2. sST2 emerged as an independent predictor of clinical outcomes in two large cohort of hospitalized COVID-19 patients, warranting further investigation to elucidate its role in disease progression and potential as a therapeutic target.

Keywords SARS-CoV-2, COVID-19, Angiogenesis, Fibrosis, ST2

¹Innovative Therapies in Hemostasis, INSERM, University Paris Cité, 75006 Paris, France. ²Hematology Department, AP-HP Centre, Georges Pompidou European Hospital, Université Paris Cité, 20 Rue Leblanc, 75015 Paris, France. ³Département d'Épidémiologie, Biostatistique et Recherche Clinique, AP-HP, Hôpital Bichat, 75018 Paris, France. ⁴IAME, INSERM, Université de Paris, 75018 Paris, France. ⁵Inserm, CHU Lille, Institut Pasteur de Lille, U1011-EGID, Université de Lille, 59000 Lille, France. ⁶Service de Maladies Infectieuses et Tropicales, AP-HP, Hôpital Bichat, 75018 Paris, France. ⁷Department of Infectious Diseases, University Hospital of Saint-Etienne, Saint-Etienne, France. ⁸CIC-INSERM 1408, University Hospital of Saint-Etienne, Saint-Etienne, France. ⁹Assistance Publique – Hôpitaux de Paris, Hôpitaux Universitaires Pitié-Salpêtrière Charles Foix, Service de Maladies Infectieuses et Tropicales, INSERM 1136, Institut Pierre Louis d'Épidémiologie et de Santé Publique, Sorbonne Université, 75013 Paris, France. ¹⁰Department of Infectious Diseases, CIC 1413, INSERM, CHU Nantes, Nantes Université, Nantes, France. ¹¹Infectious Diseases Department, CHU, Dijon, and INSERM CIC 1432, Université de Bourgogne-Franche Comté, Dijon, France. ¹²UMR-CNRS 6249 Chrono-Environnement, Department of Infectious and Tropical Diseases, CHU Besançon, Université de Franche-Comté, 25000 Besançon, France. ¹³Institut de Santé Publique, INSERM, Pôle

Recherche Clinique, Paris, France. ¹⁴ANRS, Maladies Infectieuses Émergentes, Paris, France. ¹⁵Service de Maladies Infectieuses et Tropicales, CHRU-Nancy, Université de Lorraine, 54000 Nancy, France. ¹⁶AP-HP, Service Des Maladies Infectieuses, Hôpital Bicêtre, 94270 Le Kremlin Bicêtre, France. ¹⁷AP-HP, Centre National de Référence du Paludisme, Paris, France. ¹⁸Service des Maladies Infectieuses et Tropicales, Hôpitaux Universitaires de Strasbourg, 67091 Strasbourg, France. ¹⁹Service des Maladies Infectieuses et Tropicales, Centre Hospitalier Annecy Genevois, 74374 Annecy, France. ²⁰PCCEI, Inserm, EFS, Univ Montpellier, Univ Antilles, Montpellier, France. ²¹Service des Maladies Infectieuses et Tropicales, Inserm CIC1424, CHU de Martinique, Martinique, France. ²²Service des Maladies Infectieuses et Tropicales, CHU de Nice, Nice, France. ²³U1065, Centre Méditerranéen de Médecine Moléculaire, C3M, Virulence Microbienne et Signalisation Inflammatoire, INSERM, Université Côte d'Azur, Nice, France. ²⁴Fédération Hospitalo-Universitaire OncoAge, Nice, France. ²⁵Département de Pneumologie et d'Oncologie, CHU de Nice, 06000 Nice, France. ²⁶CNRS UMR 7275, IPMC, Université Côte d'Azur, Sophia Antipolis, France. ²⁷Service de Réanimation Médicale, Groupe Hospitalier de la Région Mulhouse Sud-Alsace, Mulhouse, France. ²⁸CHU de Reims, Service de Réanimation Médicale, Université de Reims Champagne-Ardenne, Reims, France. ²⁹Service d'Immunologie et Maladies Infectieuses, AP-HP, Hôpital Henri Mondor, 94000 Créteil, France. ³⁰INSERM U955, Université Paris-Est Créteil, 94000 Créteil, France. ³¹Service de Maladies Infectieuses et Tropicales, CHU Amiens-Picardie, 80000 Amiens, France. ³²AGIR UR UPJV 4294, CURS, Université Picardie Jules Verne, 80000 Amiens, France. ³³Département de Soins Intensifs, Hospices Civils de Lyon, Hôpital Lyon-Sud Pierre-Bénite, 69000 Lyon, France. ³⁴Service de Réanimation Médicale, Hospices Civils de Lyon, Hôpital de la Croix-Rousse, 69000 Lyon, France. ³⁵CREATIS, CNRS UMR5220, INSERM U1044, INSA, Université Lyon I, 69000 Lyon, France. ³⁶Service de Réanimation-Soins Intensifs, Centre Hospitalier de Luxembourg, 1210 Luxembourg, Luxembourg. ³⁷Laboratoire de Virologie, Institut des Agents Infectieux de Lyon, Centre National de Référence des Virus Respiratoires France Sud, Hospices Civils de Lyon, 69317 Lyon, France. ³⁸Virpath, CIRI, INSERM U1111, CNRS UMR5308, ENS Lyon, Université Claude Bernard Lyon 1, 69372 Lyon, France. ³⁹Department of Internal Medicine III With Haematology, Medical Oncology, Haemostaseology, Infectiology and Rheumatology, Oncologic Center, Salzburg Cancer Research Institute - Laboratory for Immunological and Molecular Cancer Research (SCRI-LIMCR), Paracelsus Medical University Salzburg, 5020 Salzburg, Austria. ⁴⁰Service des Maladies Infectieuses et Tropicales, CHU de Toulouse, 31320 Toulouse, France. ⁴¹Institut Toulousain des Maladies Infectieuses et Inflammatoires (Infinity) INSERM UMR1291 - CNRS UMR5051, Université Toulouse III, 31320 Toulouse, France. ⁴²Département de Pneumologie, CHU d'Amiens, 80000 Amiens, France. ⁴³Intensive Care Unit, AP-HP Centre Université Paris Cité, Georges Pompidou European Hospital, 75015 Paris, France. ⁴⁴Clinic of Infectious Diseases, Hôpital Universitaire de Bruxelles (H.U.B.), 1070 Brussels, Belgium. ⁴⁵Département des Maladies Infectieuses et Tropicales, Hospices Civils de Lyon, Lyon, France. ⁴⁶Département des Maladies Infectieuses et Tropicales, CIRI, INSERM U1111, CNRS UMR5308, ENS Lyon, Université Claude Bernard Lyon 1, Lyon, France. ¹⁰⁹David M. Smadja, Clément R. Massonnaud contributed equally to the work. ¹¹⁰Aurélien Philippe, Mickael Rosa contributed equally to the work. ¹¹¹France Mentré and Annabelle Dupont contributed equally to the work. *A list of authors and their affiliations appears at the end of the paper. ✉email: david.smadja@aphp.fr

The emergence of SARS-CoV-2 sparked a global COVID-19 crisis, with the case fatality ratio dropping from 2% in February 2020 to below 0.3% in 2022¹. Beyond pulmonary entanglements culminating in acute respiratory distress syndrome (ARDS)², severe COVID-19 cases exhibit an array of extrapulmonary manifestations. These include acute kidney injury, acute cardiac damage, coagulation dysregulation, thromboembolic episodes in particularly pulmonary embolism, and disseminated microthrombi observed in several autopsy studies^{3,4}. To date, biomarker research has primarily focused on unraveling pathophysiology, with limited applications in predicting outcomes, severity, and in-hospital mortality. However, studies on inflammation, coagulopathy, and endotheliopathy suggest potential biomarkers for disease severity^{5,6}. These include hypercoagulation, evidenced by the presence of fibrin degradation products (D-dimer), which are associated markers with COVID-19 severity and outcomes⁷. Direct endothelial infection by SARS-CoV-2 remains uncertain⁸. Endotheliopathy-driven dysfunction could fuel hypercoagulation by increasing the levels of von Willebrand factor (VWF)⁹. Beyond thrombotic events, recent studies have highlighted the role of pathological angiogenesis^{3,5} which could give rise to subsequent fibrotic remodeling^{10,11}.

The objective of our study was to examine the associations between baseline levels of various circulating biomarkers linked to thromboinflammation, endothelial damage, angiogenesis, or fibrosis, and clinical outcomes in hospitalized COVID-19 patients participating in the European randomized controlled study DisCoVeRy¹². The biomarker identified in the primary analysis was tested in an independent confirmation cohort of hospitalized COVID-19 patients (SARCODO study)⁹.

Material and methods

Participants

This protocol is based on the protocol produced by the National Institute of Health for the World Health Organization (WHO), version of 03/03/2020, which further led to the Solidarity protocol of WHO. This study supports the integration of the Solidarity WHO trial worldwide. Hospitalized patients with a laboratory-confirmed SARS-CoV-2 infection were enrolled in the DisCoVeRy trial¹³, sponsored by the Institut national de la santé et de la recherche médicale (Inserm, France). The aim of this trial was to compare the effectiveness of standard of care (SoC) plus lopinavir/ritonavir (SoC + Lopi/Rito), SoC plus lopinavir/ritonavir-interferon (IFN)- β -1a (SoC + Lopi/Rito + IFB), or SoC plus hydroxychloroquine (SoC + HCQ) to SoC alone, in hospitalized patients during the first wave of the COVID-19 pandemic. Written informed consent was obtained from all participants or their legal representatives if they were unable to consent. From March 22 to June 29, 2020, a total of 603 individuals were randomly assigned to different treatment groups at 30 locations in France and two in Luxembourg. The study included adults aged 18 and older who were hospitalized due to a PCR-confirmed

SARS-CoV-2 infection, exhibiting pulmonary rales or crackles, and having a peripheral oxygen saturation of 94% or lower, or who required supplemental oxygen. Patients were randomly allocated in a 1:1:1:1 ratio to receive SoC alone, SoC + Lopiv/Rito, SoC + Lopiv/Rito + IFB, or SoC + HCQ as previously described^{12,13}. The trial was conducted in accordance with the Declaration of Helsinki and national laws and regulations, approved by the Ethics Committee (CPP Ile-de-France-III, approval #20.03.06.51 744), and declared on the clinicaltrials.gov registry (NCT04315948, first posted on 20/03/2020).

Biomarker measurements

Blood samples were collected just after inclusion/randomization and before administration of an investigational drug (baseline) in ethylenediaminetetraacetic acid (EDTA). Platelet-poor plasma (PPP) was obtained after centrifugation at $2500 \times g$ for 15 min and stored at -80°C until analysis. The D-dimer level was measured with the immunoturbidimetric STA[®]—Liatest[®] D-Di PLUS (STAGO Asnières sur Seine, France). The concentrations of nucleosomes, LDH, citrullinated Histone H3 (H3cit), CRP, and P-selectin were determined using ELISA respectively from Sigma-Aldrich (nucleosomes and LDH), Cayman Chemical (H3cit) and R&D Systems (CRP and P-selectin). The plasma concentrations of angiopoietin-1 and -2, CA 15-3/MUC-1, CD40 Ligand/TNFSF5, Ferritin, FGF-2, IL-6, PDGF-BB, PIGF, soluble Suppressor of tumorigenicity 2 (ST2; receptor of soluble IL33), VEGF-A, ICAM1/CD54, Syndecan-1, VWF, D-dimers, E-selectin, VCAM-1/CD106 and Troponin I were quantified in PPP using a Human Magnetic Luminex Assay (R&D Systems, Minneapolis, MN). Data were assessed with the Bio-Plex 200 using the Bio-Plex Manager 5.0 software (Bio-Rad, Marnes-la-Coquette, France). Cell-free DNA was measured by spectrofluorimetry at 520 nm after excitation at 480 nm with Quant-iT PicoGreen dsDNA assay (Thermo Fischer Scientific, Waltham, MA) on a SAFAS spectrophotometer (Monaco, France).

Outcomes

The two main outcomes considered were the time to hospital discharge before day 29, and the change in National Early Warning Score (NEWS-2 score) between baseline and day 15.

- The time to hospital discharge before day 29 was computed as the number of days between the randomization date, and the date of discharge from the index hospitalization, censored at day 29. Death before day 29 was treated as a competing risk of hospital discharge.
- The NEWS-2 score is a standardized clinical scoring system developed for improved detection of deterioration in acutely ill patients that has demonstrated good performance in COVID-19 patients^{14–16}.

The National Early Warning Score (NEWS) was introduced in 2012 to facilitate the early detection of clinical deterioration in acutely ill patients within NHS acute medical units¹⁷. Its primary aim was to establish a standardized system for tracking, scoring, and responding to physiological changes. The system is based on the principle that early recognition and prompt intervention significantly impact patient outcomes. Due to its effectiveness, NEWS has been widely adopted across the NHS and internationally. NEWS functions as a cumulative scoring system that assigns numerical values to routine physiological measurements recorded during patient monitoring. In 2017, an updated version, NEWS 2, was introduced, aligning its structure with the Resuscitation Council UK's ABCDE framework¹⁸. The system evaluates six key physiological parameters: respiratory rate, oxygen saturation, systolic blood pressure, pulse rate, temperature, and level of consciousness or new confusion. Each parameter is assigned a score from 0 to 3, with higher scores indicating greater deviation from normal values and an increased risk of clinical deterioration¹⁸. The change in NEWS-2 score between baseline and day 15 was computed as the difference between the scores assessed at day 15 and at baseline. For participants who died before day 15, the NEWS-2 score was imputed to worst possible value (i.e. NEWS-2 score of 20). The proportion of patients that had died by day 29 was analyzed as a post-hoc secondary outcome.

Covariates

All analyses were adjusted on four baseline covariates: (1) the ISARIC 4C mortality score, (2) the disease severity at baseline during randomization (moderate: WHO 7-point ordinal scale 3 or 4; or severe: WHO 7-point ordinal scale 5 or 6), (3) the log₁₀ D-dimer value at baseline, and (4) the treatment regimen: SoC, SoC + HCQ, SoC + Lopiv/Rito, and SoC + Lopiv/Rito + IFB. The D-dimer levels at baseline have been shown to be associated with clinical outcomes of hospitalized COVID-19 patients⁷. The disease severity at randomization and the ISARIC 4C mortality score have been selected as they are key markers of clinical severity of patients at baseline. The ISARIC 4C mortality score is a risk stratification score that predicts in-hospital mortality for hospitalized COVID-19 patients, produced by the ISARIC 4C consortium^{19,20}. Developed by the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC), this score integrates clinical, demographic, and laboratory parameters to assess disease severity. It is calculated using eight variables: age, sex, number of comorbidities (0, 1, 2 or more), respiratory rate, oxygen saturation, Glasgow Coma Scale score, blood urea level, and C-reactive protein level. Increased BMI is considered as a comorbidity. Each parameter is assigned a numerical score based on its deviation from the normal range, resulting in a total score between 0 and 21, where higher values indicate an increased risk of mortality. The stratification system categorizes patients into different risk groups to aid clinical decision-making. The classification system defines risk groups based on the total score. A low-risk classification applies to patients scoring 0 to 3, corresponding to an estimated 1.2% mortality risk. The intermediate-risk category includes scores from 4 to 8, where mortality risk increases to 9.9%. The high-risk category consists of patients with scores 9 or above, who have an estimated 48.2% mortality rate. These thresholds provide essential guidance for clinicians in triaging COVID-19 patients and optimizing medical resource allocation. The ISARIC 4C Mortality Score has been externally validated and remains one of the most

reliable predictors of mortality in hospitalized COVID-19 patients. When one of the variables needed to compute the ISARIC 4C score was missing, the missing 4C score was imputed by a single multivariate imputation using a predictor matrix comprised of the outcome variables (i.e. NEWS-2 score, time to hospital discharge, event of discharge before day 29, event of death before day 29), and the 8 variables used to compute the score. Missing values for other variables were not imputed.

Statistical analysis

The baseline concentration of the biomarkers, and other continuous variables, were described by their median value and interquartile range. Categorical variables were described by the absolute and relative frequencies. For some descriptive analyses, the ISARIC 4C score was considered categorical, using the categories defined by the ISARIC consortium: low mortality risk [0–3], intermediate mortality risk (3,8], high mortality risk (8,14], very high mortality risk (14,21]. First, associations between the log₁₀ baseline values of the biomarkers and disease severity at baseline during randomization (moderate or severe) were tested with univariate logistic regression, and the p-values, q-values (according to Storey's procedure), as well as the area under receiver operating characteristic (ROC) curves (AUC) were reported. Given that disease severity at randomization was a stratification factor in the DisCoVeRy study, and to maintain consistency with previous work, associations between baseline covariates and disease severity at randomization were evaluated using Wilcoxon rank-sum tests, Pearson's chi-squared tests, or Fisher's exact tests, as appropriate. The proportions of patients discharged from hospital by day 29, considering the competing risk of death before day 29, were estimated using the Aalen-Johansen estimator (equivalent of the Kaplan–Meier method in the competing risk setting), and compared according to disease severity at randomization using a normal approximation. Correlations coefficients between each pair of biomarkers were computed on log₁₀ values and tested using Pearson test. To assess the relationships between the four baseline covariates and clinical outcomes, we first fitted multivariate models that included only the covariates. The time to hospital discharge before day 29 was analyzed using a multivariate proportional-hazards model with a Fine and Gray approach considering the competing risk of death before day 29 (R package “survival”). The change in NEWS-2 score between baseline and day 15 was analyzed using a multivariate linear model. Then, to assess the individual association between the log₁₀ baseline concentration of the biomarkers and the two outcomes, we included each marker individually in the models along with the rest of the covariates. Finally, we fitted models to assess the association between a combination of biomarkers and the two outcomes. To do so, biomarkers individually associated with the outcomes with a p-value less than or equal to 0.20 were selected using a backward selection process, with the covariates forced in the final model. The goodness of fit of proportional-hazards models were assessed using cumulative/dynamic time-dependent AUC. The goodness of fit of the different models were compared statistically using the time-dependent AUC evaluated at t=29 (R package “timeROC”). The confidence intervals and p-values were computed using the jackknife approach. In a secondary, post-hoc analysis, the associations between the log₁₀ biomarker concentrations and the mortality by day 29 was assessed by multivariate logistic regressions, with the same approach as the other outcomes. All analyses were done using the R software, version 4.3.1.

Post-hoc analyses in SARCODO cohort

Post-hoc analyses were performed on a confirmation cohort of hospitalized patients with a laboratory-confirmed SARS-CoV-2 infection enrolled in the SARCODO cohort (NCT04624997), sponsored by the *Assistance Publique–Hôpitaux de Paris*. This confirmation cohort is a monocentric cross-sectional study of adult (> 18-years old) COVID-19 hospitalized patients in European Georges Pompidou Hospital between March 13 and June 5, 2020. Covid-19 disease severity was classified according to the WHO Working Group on the Clinical Characterization and Management of Covid-19 infection as previously described⁹. Written informed consent was obtained from all participants or their legal representatives if they were unable to consent. 103 patients for this confirmation cohort were included as previously described^{9,21}. At the time of hospital admission for suspected COVID-19, routine laboratory tests and sST2 measurements were conducted within the first 48 h. Venous blood samples were taken from patients and handled using standard laboratory procedures. The blood was collected in 0.129M trisodium citrate tubes (9NC BD Vacutainer, Plymouth, UK). Platelet-poor plasma (PPP) was prepared by centrifuging the blood twice at 2500 g for 15 min, and then stored at – 80 °C until further analysis. The plasma concentrations of sST2 were quantified in PPP using a Human Quantikine Elisa (R&D Systems, Minneapolis, MN). Demographic, clinical and biological characteristics, and clinical outcomes of patients included in SARCODO were described by their median value and interquartile range (continuous variables), and by the absolute and relative frequencies (categorical variables). The confirmation cohort of the SARCODO trial did not have the variables necessary to compute the NEWS-2 score, so the post-hoc analyses were performed only on the time to hospital discharge before day 29, defined as for the DisCoVeRy analysis. Moreover, as the treatment strategies used in DisCoVeRy were not used in SARCODO, and as the disease severity at baseline was not available, these variables were not included in the models. The biomarkers selected in the final models of DisCoVeRy were tested in SARCODO in models including ISARIC 4C-score and D-dimer, using the same statistical procedures as for the DisCoVeRy analysis.

Results

Baseline characteristics and outcomes description

Of the 603 participants randomized in the Discovery trial, 593 were evaluable for analysis. Of them, 454 had a sample collected at baseline and measured (Fig. 1 and supplementary Table S1).

Of the patients who had at least one baseline sample, 323 (71%) were men, with a median age of 63 years (IQR 54–71) and a median BMI of 27.7 kg/m² (IQR 25.2–32.0). The median time from symptom onset to hospitalization was 9 days (IQR 7–12), and 309 patients (68%) had a moderate disease severity assessed at

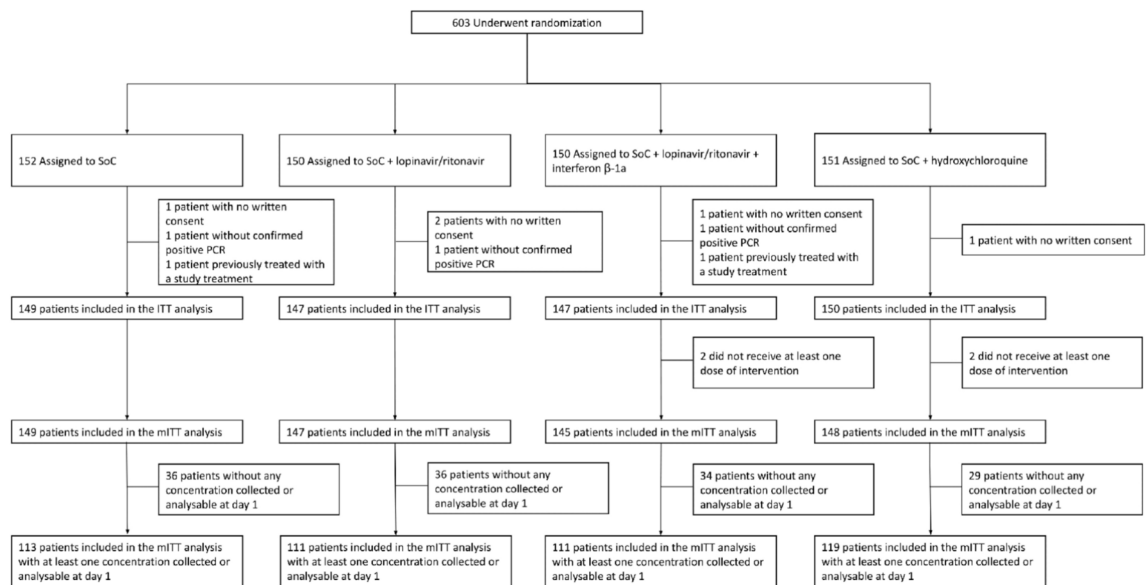


Fig. 1. Flow chart of discovery trial.

baseline, whereas 145 (32%) were severe. Among the 309 patients with moderate disease severity, 281 (91%) needed only supplemental oxygen. In contrast, of the 145 patients classified as having severe disease, 35 (24%) required non-invasive ventilation or high-flow oxygen devices, while 96 (66%) necessitated invasive ventilation or extracorporeal membrane oxygenation (ECMO). Overall, 25 patients had died by day 29 (Table 1).

The ISARIC 4C score was significantly associated with disease severity at randomization. As previously described, all biomarkers of thromboinflammation, endothelial dysfunction or fibrosis were associated with disease severity at randomization, notably D-dimer, Ang-2, syndecan-1 and soluble Suppressor of Tumorigenicity 2 (sST2) (Table 2). The two outcomes, namely time to hospital discharge before day 29 and change in NEWS-2 score between baseline and day 15, were both associated with disease severity at randomization (Table 1). The results of the multivariate models that included only the covariates showed that a higher baseline log10 concentration of D-dimer, a greater ISARIC 4C score, and a more severe disease at baseline were associated with lower rates of hospital discharge before day 29 (supplementary Figures S1A and S2). On the other hand, a greater ISARIC 4C score and a more severe disease at baseline were associated with lower changes of NEWS-2 score between baseline and day 15 (supplementary Figures S1B and S3).

Baseline soluble ST2 is the best biomarker associated with the time to hospital discharge before day 29 and NEWS-2 score, in DisCoVeRy

We then explored the association between the log10 baseline concentrations of each of the 21 biomarkers and the two outcomes in Discovery (Table 3). Fourteen and eight biomarkers had a *p*-value less than or equal to 0.20 for the association with the time to hospital discharge before day 29, and the change in NEWS-2 score between baseline and day 15, respectively. Figure 2A,B show the results of the final model after backward selection.

Among the 14 biomarkers included in the selection of the model of time to hospital discharge before day 29, only sST2 (aHR 0.25; 95% CI [0.16–0.39]; $p < 10^{-8}$) and the nucleosomes (aHR 0.63; 95% CI [0.48–0.81]; $p < 10^{-3}$) remained in the model. Higher baseline levels of these markers were significantly associated with lower rates of hospital discharge before day 29, after having adjusted for the ISARIC 4C score, the disease severity at baseline, the treatment regimen, and the baseline D-dimer level (Figs. 2A, 3A and S4).

Regarding the goodness of fit, the final model had a time-dependent AUC at $t = 29$ of 86% (95% CI [82–89]), while the model containing only the covariates had a time-dependent AUC at $t = 29$ of 83% (95% CI [79–87]), which was statistically significantly lower (p -value = 0.047) (Fig. 4A).

Among the 8 biomarkers included in the model of the change in NEWS-2 score between baseline and day 15, only sST2 remained, higher baseline levels of the marker being significantly associated with lower changes of NEWS-2 score between baseline and day 15 (adjusted beta 4.75; 95% CI [2.92–6.57]; $p < 10^{-6}$) (Figs. 2B and 3B). The final model had a goodness of fit (adjusted R-squared) of 0.09 (95% CI [0.04–0.14]), while the model with only the covariates had a goodness of fit of 0.037 (95% CI [0.004–0.072]). The post-hoc analysis of mortality by day 29 showed that the association with the baseline concentrations of sST2 was statistically significant when adjusted for the other baseline covariates (p -value $< 10^{-2}$, supplementary Figure S6). Scatterplots were drawn to visualize the statistically significant (p -value < 0.05) correlations between ST2/IL-33R and the other biomarkers in DisCoVeRy (Fig. 5). Interestingly, sST2 was correlated with circulating DNA, angiogenic biomarker PlGF or endotheliopathy markers Syndecan-1 and VCAM-1 (Pearson's coefficients of 0.46 for DNA, 0.41 for PlGF, 0.40 for Syndecan-1 and 0.37 for VCAM-1).

| Characteristic | N | Overall, N = 454 | Disease severity at randomization | | p-value |
|---|-----|---------------------|-----------------------------------|---------------------|---------------------|
| | | | Moderate, N = 309 | Severe, N = 145 | |
| Age, years | 454 | 63 (54, 71) | 63 (53, 72) | 64 (56, 71) | 0.52 |
| Sex male | 454 | 323 (71%) | 217 (70) | 106 (73%) | 0.53 |
| BMI, kg/m ² | 398 | 27.7 (25.2, 32.0) | 27.2 (25.0, 31.2) | 28.7 (26.0, 34.4) | 0.007 |
| Days from symptoms onset to randomization | 451 | 9.0 (7.0, 12.0) | 9.0 (7.0, 12.0) | 9.0 (7.0, 12.0) | 0.78 |
| Ventilatory support at randomization | 454 | | | | < 10 ⁻⁶ |
| Room air | | 32 (7%) | 30 (10%) | 2 (1%) | |
| Oxygen support | | 331 (73%) | 275 (89%) | 56 (39%) | |
| Invasive Mechanical Ventilation | | 91 (20%) | 4 (1%) | 87 (60%) | |
| WHO 7-points ordinal scale at baseline | 454 | | | | < 10 ⁻⁶ |
| (3) Hospitalized, not requiring supplemental oxygen | | 11 (2%) | 11 (4%) | 0 (0%) | |
| (4) Hospitalized, requiring supplemental oxygen | | 295 (65%) | 281 (91%) | 14 (10%) | |
| (5) Hospitalized, on non-invasive ventilation or high flow oxygen devices | | 46 (10%) | 11 (4%) | 35 (24%) | |
| (6) Hospitalized, on invasive mechanical ventilation or ECMO | | 102 (22%) | 6 (2%) | 96 (66%) | |
| ISARIC 4C score (continuous) ^a | 454 | 11 (9, 13) | 10 (8, 12) | 12 (10, 14) | < 10 ⁻³ |
| ISARIC 4C score (categorical) ^a | 454 | | | | 0.002 |
| Low: [0,3] | | 4 (0.9%) | 4 (1.3%) | 0 (0%) | |
| Intermediate: (3,8] | | 105 (23%) | 83 (27%) | 22 (15%) | |
| High: (8,14] | | 303 (67%) | 201 (65%) | 102 (70%) | |
| Very High: (14,21] | | 42 (9.3%) | 21 (6.8%) | 21 (14%) | |
| NEWS2 score at baseline | 454 | 9 (7, 12) | 8 (6, 10) | 12 (10, 15) | < 10 ⁻⁶ |
| NEWS2 score at day 15 | 454 | 5 (2, 10) | 3 (1, 6) | 11 (7, 14) | < 10 ⁻⁶ |
| Change in NEWS2 from baseline to day 15 | 454 | - 4 (- 7, 0) | - 4 (- 7, - 2) | - 2 (- 5, 2) | < 10 ⁻⁴ |
| Discharged from hospital at day 29 ^b | 454 | 65.2% [61.0%–69.8%] | 81.2% [76.9%–85.7%] | 31.7% [25.0%–40.3%] | < 10 ⁻¹⁵ |
| Number of deaths by day 29 | 454 | 25 (6%) | 15 (5%) | 10 (7%) | 0.37 |

Table 1. Patient characteristics at baseline, overall, and by disease severity at randomization, in DisCoVeRy. Data is n (%) or median (IQR). ^aAt least one variable needed to compute the ISARIC 4C score was missing in 89 participants. Missing scores have been imputed by a single multivariate imputation. ^bData is the proportion of patients discharged from hospital at day 29. The 95% confidence interval and the *p*-value for the difference of proportions were estimated using the Aalen-Johansen estimator.

Association between sST2 and time to hospital discharge before day 29 in SARCODO

Demographic, clinical and biological characteristics, and clinical outcomes of the 103 SARCODO participants are presented in Supplementary Table S2. As the nucleosomes’ baseline concentrations and the NEWS-2 score were not available in the SARCODO data, we only explored the association between sST2 and time to hospital discharge before day 29. The median value of sST2 at baseline was 41,918 pg/mL (IQR [22,321–106,059]). Our findings indicated that elevated sST2 levels were significantly associated with lower rates of hospital discharge before day 29, even after adjusting for the ISARIC 4C score and baseline D-dimer levels: aHR 0.13 (95% CI [0.06–0.27], *p*-value < 10⁻⁷) (Fig. 2C).

Regarding the goodness of fit of the model, the SARCODO model with sST2 had a time-dependent AUC at *t* = 29 of 92% (95% CI [86–97]), while the SARCODO model with only ISARIC and D-dimer had a time-dependent AUC at *t* = 29 of 79% (95% CI [68–89]), which was statistically significantly lower (*p*-value = 0.011) (Fig. 4B). Even a model with sST2 alone had a better time-dependent AUC at *t* = 29 than a model with ISARIC + D-dimer, with 90% (95% CI [85–96], *p*-value = 0.041).

Discussion

In this study, we address the relationship between SARS-CoV-2-mediated thromboinflammation, endotheliopathy, angiogenesis and fibrosis at hospital admission and the severity of COVID-19 in a large cohort of well-characterized patients. Among the top five biomarkers associated with outcomes, a consistent pathophysiological significance is observed across both primary endpoints. DNA, particularly its association with nucleosome levels, underscores the role of Damage-Associated Molecular Patterns (DAMPs) in COVID-19 pathology^{22,23}. DAMPs, including nucleosomes and extracellular DNA, contribute to thromboinflammation and endotheliopathy, two key mechanisms driving severe disease progression^{5,24}. The release of DNA and nucleosomes into circulation, often due to cell death and immune activation, can trigger inflammatory cascades and coagulation disturbances, exacerbating vascular dysfunction and organ injury in severe COVID-19 cases^{22–24}. Furthermore, VCAM-1 and von Willebrand factor (vWF) have emerged as critical biomarkers of endothelial dysfunction, reinforcing the connection between endotheliopathy and disease severity^{5,9,25,26}. Recent scientific investigations have highlighted their relevance, particularly in the context of COVID-19-associated coagulopathy and microvascular complications²⁶. Prior studies, including our own, have demonstrated a strong link between inflammatory endotheliopathy and COVID-19 mortality, further validating the role of endothelial damage as

| Biomarker | N | Overall, N = 454 | Disease severity at randomization | | p-value ^a | q-value ^a | AUC ^b |
|-------------------------------------|-----|---------------------------|-----------------------------------|---------------------------|----------------------|----------------------|------------------|
| | | | Moderate, N = 309 | Severe, N = 145 | | | |
| Syndecan-1/CD138 (pg/mL) | 432 | 7936 (5885–10,717) | 7043 (5383–9065) | 10,813 (7913–13,230) | < 10 ⁻¹⁵ | < 10 ⁻¹⁴ | 0.77 [0.72–0.82] |
| sST2/IL-33R (pg/mL) | 449 | 41,665 (26,035–76,828) | 33,799 (22,547–55,589) | 70,813 (39,860–118,994) | < 10 ⁻¹³ | < 10 ⁻¹² | 0.74 [0.70–0.79] |
| DNA (ng/mL) | 400 | 0.34 (0.26–0.47) | 0.31 (0.25–0.40) | 0.46 (0.36–0.64) | < 10 ⁻¹⁰ | < 10 ⁻¹⁰ | 0.75 [0.70–0.80] |
| Angiopoietin-2 (pg/mL) | 449 | 3866 (3027–4906) | 3561 (2810–4393) | 4576 (3704–5873) | < 10 ⁻⁸ | < 10 ⁻⁸ | 0.71 [0.66–0.76] |
| CRP (mg/L) | 449 | 167 (87–264) | 136 (66–207) | 253 (169–482) | < 10 ⁻⁸ | < 10 ⁻⁸ | 0.73 [0.68–0.78] |
| P-selectin (pg/mL) | 449 | 32,237 (23,031–51,882) | 28,646 (20,875–40,928) | 44,071 (31,021–67,961) | < 10 ⁻⁷ | < 10 ⁻⁷ | 0.69 [0.63–0.74] |
| Nucleosomes (arb. unit) | 424 | 0.31 (0.16–0.56) | 0.23 (0.14–0.44) | 0.46 (0.31–0.82) | < 10 ⁻⁷ | < 10 ⁻⁷ | 0.71 [0.66–0.76] |
| FGF basic/FGF2/bFGF (pg/mL) | 449 | 146 (112–180) | 134 (100–175) | 161 (140–202) | < 10 ⁻⁷ | < 10 ⁻⁷ | 0.67 [0.62–0.72] |
| D-dimer Liatest (ng/mL) | 448 | 1460 (1066–2281) | 1268 (1010–1909) | 1875 (1268–4147) | < 10 ⁻⁷ | < 10 ⁻⁶ | 0.68 [0.63–0.73] |
| MPO-DNA complexes (arb. unit) | 402 | 0.74 (0.38–1.60) | 0.64 (0.30–1.25) | 1.14 (0.57–2.07) | < 10 ⁻⁵ | < 10 ⁻⁵ | 0.65 [0.60–0.71] |
| VEGF (pg/mL) | 449 | 99 (70–142) | 93 (67–127) | 120 (89–166) | < 10 ⁻⁵ | < 10 ⁻⁵ | 0.64 [0.59–0.69] |
| LDH activity (arb. unit) | 435 | 181 (118–260) | 164 (106–234) | 228 (152–311) | < 10 ⁻⁴ | < 10 ⁻⁴ | 0.66 [0.60–0.71] |
| PlGF (pg/mL) | 448 | 109 (89–137) | 104 (82–131) | 119 (100–148) | < 10 ⁻⁴ | < 10 ⁻⁴ | 0.63 [0.58–0.68] |
| CA 15–3/MUC-1 (pg/mL) | 449 | 28 (14–60) | 27 (14–51) | 37 (16–85) | < 10 ⁻³ | < 10 ⁻² | 0.58 [0.52–0.64] |
| H3cit (ng/mL) | 424 | 2.8 (1.6–4.6) | 2.5 (1.4–4.2) | 3.4 (2.1–5.7) | < 10 ⁻² | < 10 ⁻² | 0.61 [0.55–0.67] |
| CD40 Ligand/TNFSF5 (pg/mL) | 449 | 7205 (5581–9373) | 6955 (5277–9022) | 8171 (6209–9851) | < 10 ⁻² | < 10 ⁻² | 0.61 [0.55–0.66] |
| PDGF-BB (pg/mL) | 449 | 1853 (962–3765) | 1638 (876–3217) | 2228 (1281–4332) | < 10 ⁻² | < 10 ⁻² | 0.59 [0.53–0.64] |
| VCAM-1/CD106 (ng/mL) | 414 | 4150 (2683–5913) | 4025 (2411–5679) | 4712 (3029–6404) | < 10 ⁻² | 0.01 | 0.58 [0.52–0.63] |
| Von Willebrand factor-A2 (pg/mL) | 431 | 3495 (2619–4580) | 3379 (2580–4379) | 3801 (2790–4981) | 0.03 | 0.04 | 0.58 [0.52–0.63] |
| E-selectin/CD62E (pg/mL) | 432 | 31,323 (24,182–40,076) | 30,503 (23,872–39,431) | 33,339 (26,794–43,139) | 0.03 | 0.04 | 0.57 [0.52–0.63] |
| Ratio Angiopoietin-2/Angiopoietin-1 | 449 | 0.55 (0.27–1.15) | 0.56 (0.25–0.97) | 0.54 (0.30–1.37) | 0.13 | 0.15 | 0.46 [0.40–0.52] |
| Angiopoietin-1 (pg/mL) | 449 | 7238 (3813–14,248) | 6944 (3577–14,124) | 7756 (4110–15,180) | 0.28 | 0.31 | 0.53 [0.47–0.59] |
| Ferritin (ng/mL) | 449 | 678 (444–1734) | 642 (405–1703) | 768 (493–1824) | 0.39 | 0.42 | 0.56 [0.50–0.61] |
| ICAM1/CD54 (pg/mL) | 431 | 271,074 (201,451–432,876) | 273,154 (200,279–421,171) | 269,584 (208,585–434,057) | 0.46 | 0.48 | 0.48 [0.42–0.54] |
| Cardiac Troponin I/cTNI (pg/mL)* | 449 | 5.86 (5.86–5.86) | 5.86 (5.86–5.86) | 5.86 (5.86–5.86) | 0.77 | 0.77 | 0.51 [0.48–0.54] |

Table 2. Baseline values of the biomarkers, overall, and according to disease severity at randomization in DisCoVeRy. Data is median (IQR). Arb. Unit, arbitrary unit. *409 samples are below the limit of detection (LOD) and were imputed to half the LOD (5.86 pg/mL). ^aUnivariate logistic regression on the log10 transformed biomarkers values (AUC: area under ROC curve). Q-values computed according to Storey's procedure.

a key driver of adverse outcomes in critically ill patients^{9,25,27}. This growing body of evidence strengthens the understanding of DAMPs, nucleosomes, and endotheliopathy as interconnected pathophysiological pathways that contribute to the prothrombotic and inflammatory state observed in severe COVID-19^{5,24}. Results from the panel of 21 biomarkers tested on COVID-19 inpatients included in the DisCoVeRy trial establish that the stronger association between a biomarker and clinical outcomes was sST2 blood levels. DNA levels have been found significantly associated to outcomes. However, sST2 appears to be more specifically associated with outcomes, inflammatory and endothelial dysregulation seen in COVID-19. In our study, sST2 demonstrated stronger predictive performance for the two primary outcomes compared to DNA. This difference may be attributed to sST2's role as a soluble receptor involved in the IL-33/ST2 axis, a pathway critically implicated in inflammation and fibrosis. Indeed, patients with elevated sST2 levels at baseline, regardless of the disease severity at baseline, were less likely to have recovered from initial clinical deterioration by day 15 (assessed by the NEWS-2 score), and less likely to have been discharged from hospital before day 29.

ST2, the soluble form of IL-33 receptor is expressed across various cell types, such as macrophages, neutrophils, lymphocytes, endothelial cells, cardiomyocytes, osteoclasts, osteoblasts, and adipocytes²⁸. Recent years have witnessed extensive deliberation over the role of sST2 as a diagnostic and prognostic biomarker across several clinical conditions, such as acute and chronic heart failure (HF), myocardial fibrosis²⁹ and diabetic and critical limb ischemia³⁰. The lungs are a significant source of sST2 in heart failure and appear to be actively involved in the pathological response associated with ST2²⁹. Additionally, the role of sST2 has also been proposed in pulmonary diseases such as acute respiratory distress syndrome³¹ or idiopathic pulmonary fibrosis^{32,33} by contributing to inflammation and fibrotic processes^{34,35}. Regarding COVID-19, sST2/IL-33 axis has been proposed as a relevant predictive factor for severity, intensive care unit direct admission and in-hospital mortality in COVID-19 patients but also immunization level after SARS-CoV-2 infection^{36–40}. However, the various associations between sST2 and preexisting comorbidities could act as a confounding factor in our analysis of the relationship between ST2 and clinical outcomes. To address this, our models incorporate the ISARIC 4C score, which accounts for the number of preexisting comorbidities. This adjustment strengthens the validity of our findings and supports the potential of ST2 as a robust biomarker in COVID-19. Finally, a correlation has been observed between elevated soluble ST2 levels and high scores of Endothelial Activation and

| Time to hospital discharge before day 29 | | | Change in NEWS-2 score between baseline and day 15 | | |
|--|---------------------|----------------------|--|--------------------|----------------------|
| Variable | p-value | q-value ^a | Variable | p-value | q-value ¹ |
| ST2/IL-33R | < 10 ⁻¹⁰ | < 10 ⁻⁹ | ST2/IL-33R | < 10 ⁻⁶ | < 10 ⁻⁵ |
| Nucleosomes | < 10 ⁻⁵ | < 10 ⁻⁴ | Nucleosomes | 0.01 | 0.07 |
| DNA | < 10 ⁻⁵ | < 10 ⁻⁴ | VCAM-1/CD106 | 0.01 | 0.07 |
| Von Willebrand factor-A2 | < 10 ⁻⁴ | < 10 ⁻³ | Von Willebrand factor-A2 | 0.01 | 0.07 |
| VCAM-1/CD106 | < 10 ⁻³ | < 10 ⁻² | DNA | 0.04 | 0.17 |
| LDH activity | 0.02 | 0.05 | MPO-DNA | 0.13 | 0.46 |
| MPO-DNA | 0.05 | 0.16 | PIGF | 0.18 | 0.50 |
| Syndecan-1/CD138 | 0.08 | 0.20 | FGF basic/FGF2/bFGF | 0.19 | 0.50 |
| PIGF | 0.11 | 0.25 | Cardiac Troponin I/cTNI | 0.22 | 0.50 |
| ICAM/CD54 | 0.15 | 0.30 | PDGF-BB | 0.25 | 0.52 |
| P-selectin | 0.16 | 0.30 | LDH activity | 0.30 | 0.58 |
| Ferritin | 0.18 | 0.30 | VEGF | 0.34 | 0.58 |
| PDGF-BB | 0.19 | 0.30 | H3cit | 0.36 | 0.58 |
| FGF basic/FGF2/bFGF | 0.20 | 0.30 | P-selectin | 0.39 | 0.58 |
| Angiopoietin-2/Angiopoietin-1 | 0.22 | 0.30 | ICAM/CD54 | 0.44 | 0.61 |
| H3cit | 0.35 | 0.45 | Angiopoietin-2/Angiopoietin-1 | 0.61 | 0.78 |
| CA15-3/MUC-1 | 0.46 | 0.54 | E-selectin/CD62E | 0.63 | 0.78 |
| E-selectin/CD62E | 0.47 | 0.54 | Ferritin | 0.73 | 0.81 |
| VEGF | 0.63 | 0.67 | Syndecan-1/CD138 | 0.73 | 0.81 |
| CD40 Ligand/TNFSF5 | 0.64 | 0.67 | CA15-3/MUC-1 | 0.95 | 0.99 |
| Cardiac Troponin I/cTNI | 0.83 | 0.83 | CD40 Ligand/TNFSF5 | 0.99 | 0.99 |

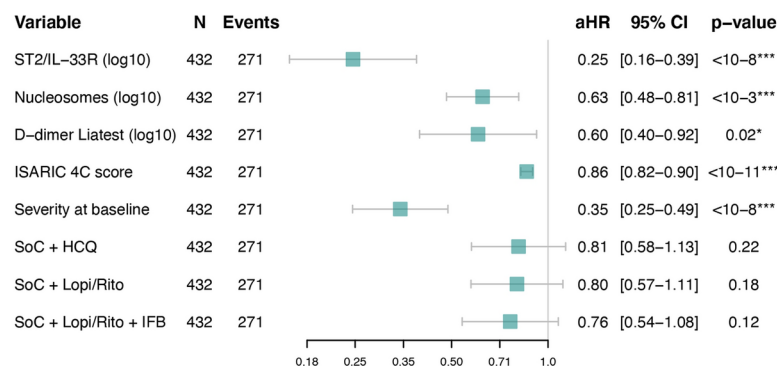
Table 3. Results of the individual analyses of the association between the log10 baseline concentrations of each biomarker and the two outcomes in DisCoVeRy. ^aQ-values computed according to Storey’s procedure.

Stress Index (EASIX) in COVID-19 cases⁴¹. This work indeed proposed a link between endothelial activation and ST2 regulation in COVID-19.

In the large DisCoVeRy clinical trial, sST2 was a stronger predictor of severity and time to hospital discharge than endotheliopathy biomarkers. Nonetheless, unraveling the precise mechanism and cell origin in this context proves to be unattainable. Given that endotheliopathy^{4,6} is a focal point in the pathophysiology of COVID-19, and considering the identified associations between sST2 and angiogenic or endotheliopathy markers in the current study, it is crucial to acknowledge the connection between vasculopathy and sST2/IL-33 axis. ST2 has been described to be crucial for revascularization after hind limb ischemia in mice. Indeed, laser doppler imaging showed that hindlimb blood flow remained severely impaired in ST2^{-/-} mice associated with reduced neovascularization in the gastrocnemius muscle and impaired neovascularization in a Matrigel implant model⁴². In addition, the expression of the angiogenic gene VEGF-A was more regulated in ST2^{-/-} mice than in WT mice⁴². This result is interesting regarding COVID-19 and its long-term complications. Indeed, we recently described that VEGF-A was increased during follow-up post COVID-19 and its increase was related to impairment in the diffusing capacity of the lung for carbon monoxide and radiological sequelae¹¹. The link between ST2 and endotheliopathy and vasculopathy is of interest. The reason is that intussusceptive angiogenesis and lobular micro-ischemia might be associated with distinctive forms of fibrotic interstitial lung disease that contribute to long COVID-19¹⁰, therefore linking ST2 and endotheliopathy/vasculopathy.

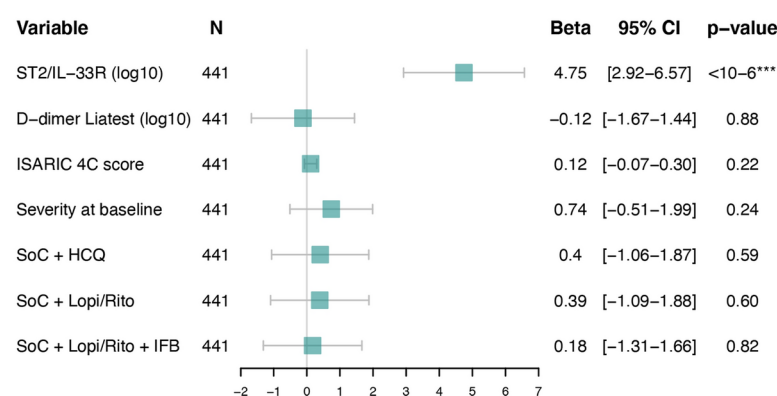
Understanding the sequence of pathophysiology regarding respiratory sequelae could help researchers to appreciate and identify new therapeutic approaches for SARS-CoV-2 infection. First, several strategies have been proposed to block the ST2/IL33 axis in various clinical conditions. Anti-ST2-conjugated nanoparticles have been shown to control lung inflammation by reducing the ability of group 2 innate lymphoid cells to produce IL-5 and IL-13, thereby reducing CD4⁺T cells⁴³. Targeting the IL33-ST2 axis with monoclonal antibodies has been proposed as an alternative treatment for cancer⁴⁴ and organ fibrosis⁴⁵. Exploring additional treatment approaches beyond antiviral medications and steroids is essential for individuals with severe COVID-19, especially in the case of immunocompromised patients, who continue to experience elevated morbidity and mortality rates. Blocking ST2 could be a potential approach and should to be tested in appropriate animal models and preclinical studies. Second, fibrosis may be linked to micro-ischemia and angiogenesis remodeling processes that are observed in COVID-19¹⁰. If so, blocking angiogenesis-induced micro-ischemia and fibrotic processes with anti-angiogenic drugs could help to stop the progression of respiratory sequelae.

Thus, despite being a non-specific inflammatory biomarker^{46,47}, sST2 exhibited strong predictive value for hospitalization duration. The unique aspect of sST2 lies in its involvement in both systemic inflammation and endotheliopathy, two hallmarks of severe COVID-19⁵. Unlike other biomarkers, sST2 reflects a broader spectrum of pathophysiological changes, including endothelial activation, fibrosis, and vascular remodeling. These processes are critical in determining clinical deterioration and recovery timelines in COVID-19. Moreover, our statistical analysis showed that sST2 had a higher discriminative performance compared to other biomarkers,



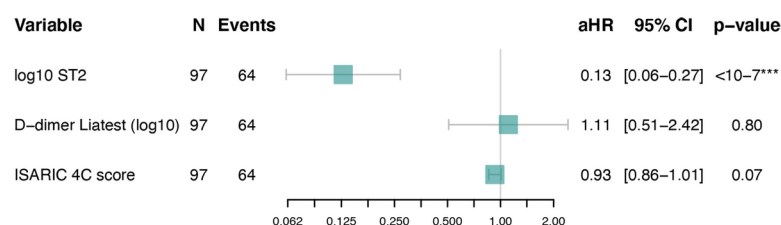
Final multivariate models with association between the log₁₀ baseline concentration of the selected biomarkers and outcomes:

A- Association between the log₁₀ baseline concentration of the selected biomarkers and the time to hospital discharge before day 29, and



Final multivariate models with association between the log₁₀ baseline concentration of the selected biomarkers and outcomes:

B- Association between the log₁₀ baseline concentration of the selected biomarkers and the change in NEWS2 between baseline and day 15, adjusted on four other baseline covariates in DisCoVeRy.



Final multivariate models with association between the log₁₀ baseline concentration of the selected biomarkers and outcomes:

C- Confirmation cohort: Association between the log₁₀ baseline concentration of sST2 and the time to hospital discharge before day 29 in SARCODO study.

Fig. 2. Final multivariate models with association between the log₁₀ baseline concentration of the selected biomarkers and outcomes.

as evidenced by its superior AUC values. This strengthens the case for sST2 as a key prognostic biomarker in COVID-19. However, this study has certain limitations. Although the multivariate models were adjusted for covariables that were strongly associated with the outcomes, we cannot exclude the possibility of residual confusion arising from unmeasured variables. In addition, given the exploratory nature of this study, the models were fit on the entire dataset; we did not employ training and validation sets. Although post-hoc analyses of an external cohort of hospitalized COVID-19 patients (SARCODO) confirmed the strong association between

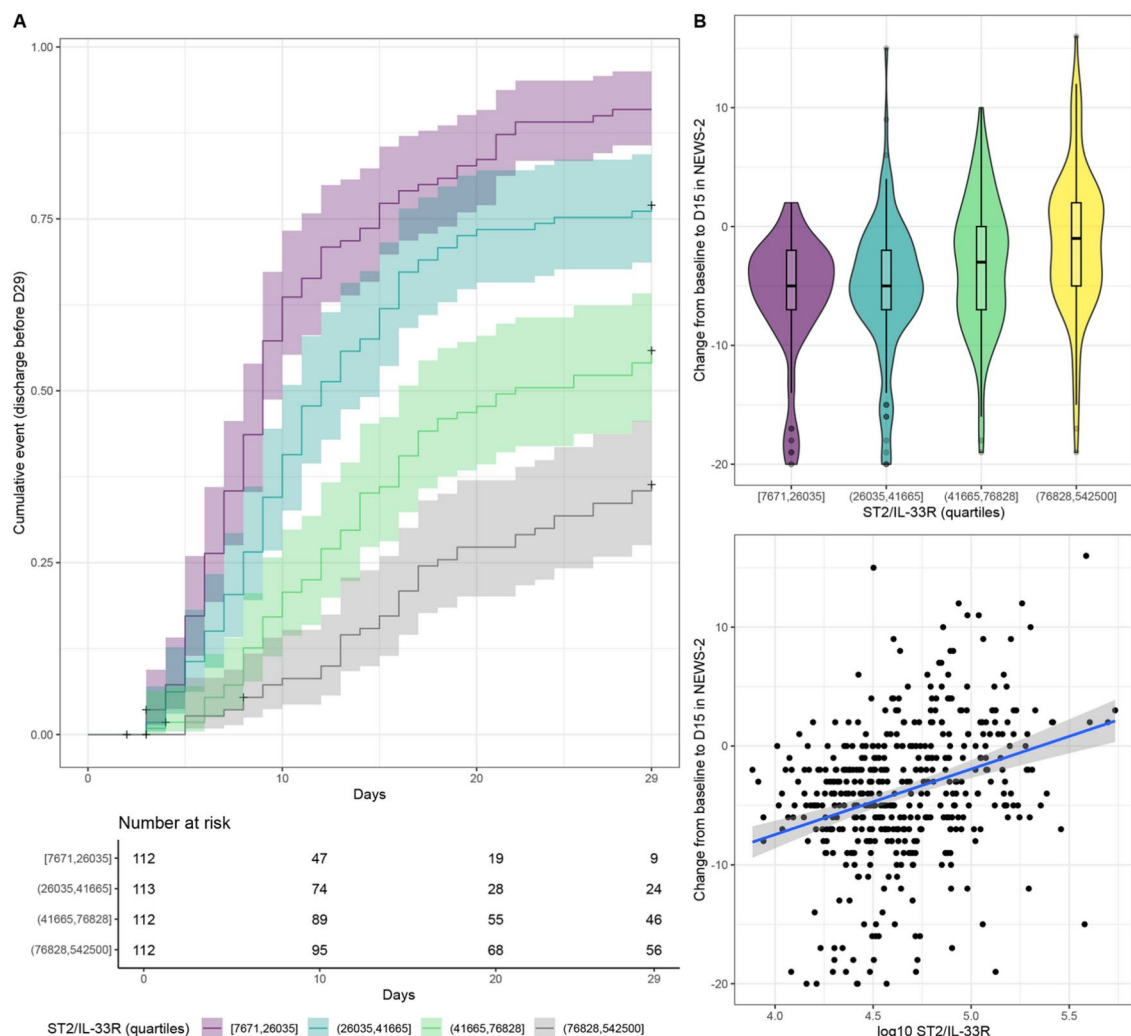


Fig. 3. Association between ST2/IL-33R values and (A) the time to hospital discharge before day 29, (B) the change in NEWS2 between baseline and day 15, in DisCoVeRy.

sST2 and time to hospital discharge before day 29, further studies are needed to obtain external validation of our findings. Finally, this study has been realized during the first wave of pandemic and we need further studies to ascertain whether this holds true for all variants of SARS-CoV-2.

All in all, this randomized controlled trial unequivocally establishes a robust association between circulating sST2 levels and clinical severity upon hospitalization. Remarkably, sST2 emerges as the best biomarker for predicting the time to hospital discharge. Our study, pioneering in its evaluation of an extensive spectrum of biomarkers within a large cohort, not only positions sST2 as a pivotal biomarker but also suggests its potential as an active participant in the pathophysiology of COVID-19. The prospect of blocking sST2 emerges as a groundbreaking therapeutic avenue, offering a new and promising approach to treating individuals afflicted with severe SARS-CoV-2 infection.

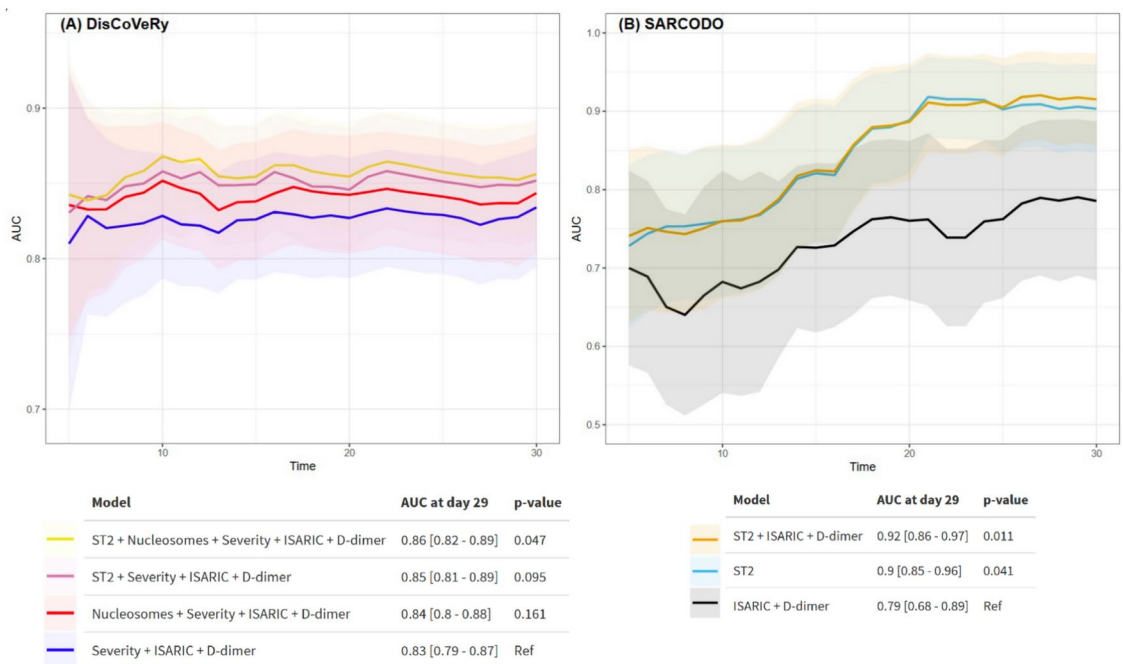


Fig. 4. Time-dependent areas under ROC curve for the proportional-hazards models of time to hospital discharge before day 29 for different models with **(A)** DisCoVeRy study. **(B)** Confirmation cohort: SARCODO study. All models for DisCoVeRy are also adjusted for the treatment strategy.

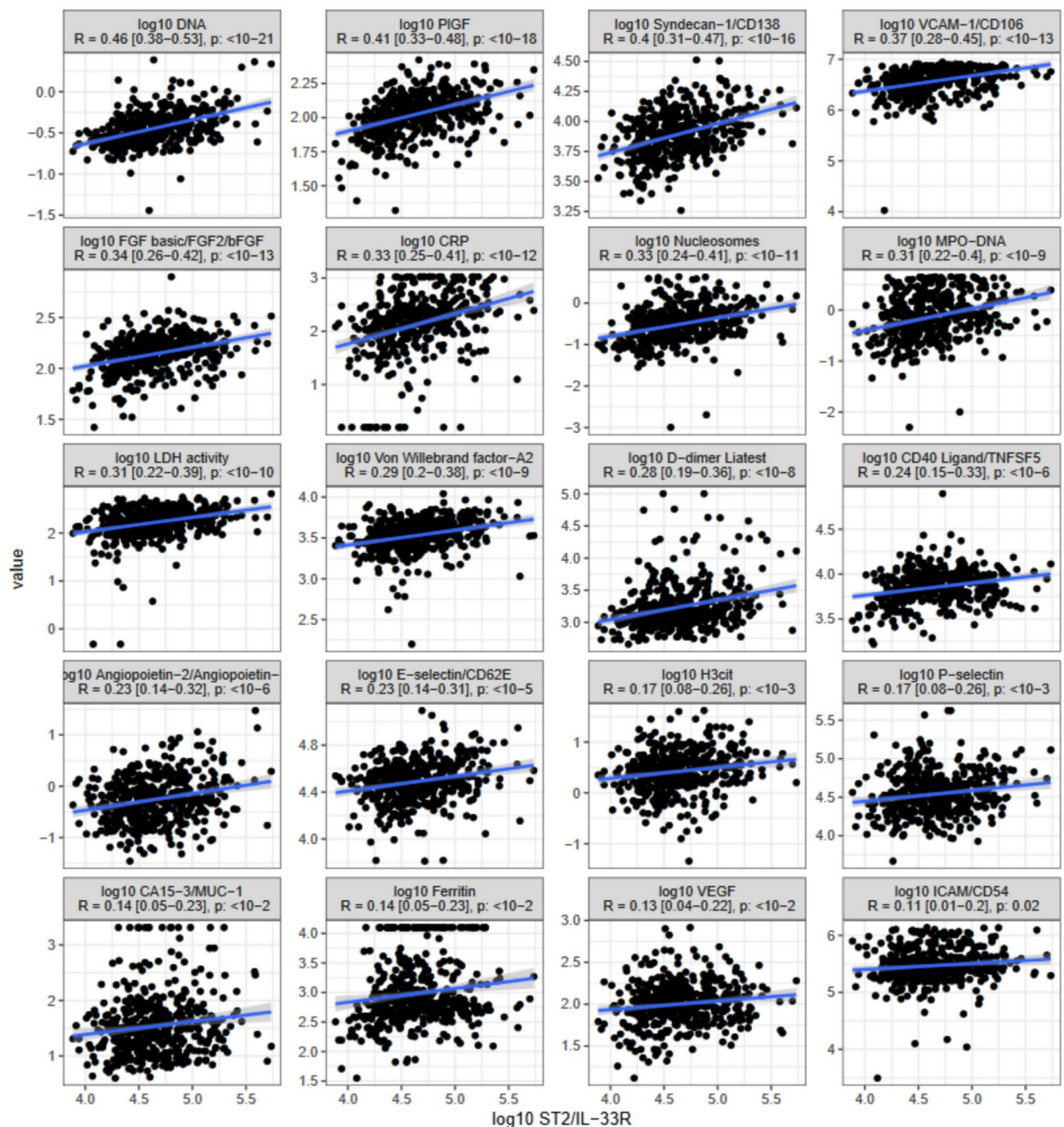


Fig. 5. Scatterplots of significant correlations ($P < 0.05$) between \log_{10} ST2/IL-33R (x-axis) and \log_{10} of biomarkers concentrations (y-axis), in DisCoVeRy.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Author contributions

All the undersigning authors have substantially contributed to the paper. All other authors included patients, reviewed all patients' characteristics, interpreted data, drafted and revised the manuscript, and approved the final version. All authors declare that the submitted work is original and has not been published before (neither in English nor in any other language) and that the work is not under consideration for publication elsewhere. David M. Smadja: PI of SARCODO trial, designed the present study, performed and/or analyzed the data and wrote the manuscript Clément R. Massonnaud: designed the present study, performed and/or analyzed the data and wrote the manuscript Aurélien Philippe: performed and/or analyzed the data Mickael Rosa: performed and/or analyzed the data Sophie Luneau: performed and/or analyzed the data Antoine Rauch: performed and/or analyzed the data Nathan Peiffer-Smadja: PI of Discovery trial, inclusion of patients Amandine Gagneux-Brunon: inclusion of patients Julien Poissy: inclusion of patients Maxime Gruest: performed and/or analyzed the data Alexandre Ung: performed and/or analyzed the data Valérie Pourcher inclusion of patients François Raffi inclusion of patients Lionel Piroth inclusion of patients Kévin Bouiller inclusion of patients Hélène Esperou work on Discovery data base Christelle Delmas work on Discovery data base Drifa Belhadi inclusion of patients Alpha Diallo inclusion of patients Juliette Saillard inclusion of patients Aline Dechanet inclusion of patients Noémie Mercier inclusion of patients Axelle Dupont inclusion of patients François-Xavier Lescure inclusion of patients François Goehringer inclusion of patients Stéphane Jaureguiberry inclusion of patients François Danion inclusion of patients Violaine Tolsma inclusion of patients André Cabie inclusion of patients Johan Courjon inclusion of patients Sylvie Leroy inclusion of patients Joy Mootien inclusion of patients Bruno Mourvillier inclusion of patients Sébastien Gallien inclusion of patients Jean-Philippe Lanoix inclusion of patients Elisabeth Botelho-nevers inclusion of patients Florent Wallet inclusion of patients Jean-Christophe Richard inclusion of patients Jean Reuter inclusion of patients Alexandre Gaymard inclusion of patients Richard Greil inclusion of patients Guillaume Martin-Blondel inclusion of patients Claire Andrejak inclusion of patients Yazdan Yazdanpanah: PI of Discovery trial, designed the present study, performed and/or analyzed the data and wrote the manuscript Charles Burdet: PI of Discovery trial, designed the present study, performed and/or analyzed the data and wrote the manuscript Jean-Luc Diehl: PI of SARCODO trial, designed the present study, performed and/or analyzed the data and wrote the manuscript Maya Hites: PI of Discovery trial, designed the present study, performed and/or analyzed the data and wrote the manuscript Florence Ader: PI of Discovery trial, designed the present study, performed and/or analyzed the data and wrote the manuscript Sophie Susen: designed the present study, performed and/or analyzed the data and wrote the manuscript France Mentré: PI of Discovery trial, designed the present study, performed and/or analyzed the data and wrote the manuscript Annabelle Dupont: designed the present study, performed and/or analyzed the data and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to D.M.S.

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the Discovery Study Group

Sandrine Couffin-Cadièrgues¹³, Hélène Esperou⁴⁷, Bernd Lamprecht⁴⁸, Michael Joannidis⁴⁹, Alexander Egle³⁹, Richard Greil⁵⁰, Antoine Altdorfer⁵¹, Vincent Fraipont⁵², Leila Belkhir⁵³, Maya Hites⁵⁴, Gil Verschelden⁵⁵, Violaine Tolsma⁵⁶, David Bougon⁵⁷, Agathe Delbove⁵⁸, Marie Gousseff⁵⁹, Nadia Saidani⁶⁰, Guilhem Wattedamps⁵⁸, Félix Djossou⁶¹, Loïc Epelboin⁶², Jean-Philippe Lanoix⁶³, Pierre-Alexandre Roger⁶⁴, Claire Andrejak⁶³, Yoann Zerbib⁶⁵, Kevin

Bouiller⁶⁶, Catherine Chirouze⁶⁷, Jean-Christophe Navellou⁶⁸, Alexandre Boyer⁶⁵, Charles Cazanave⁶⁵, Alexandre Duvignaud⁶⁹, Didier Gruson⁷⁰, Denis Malvy⁷¹, Henry Lessire⁵⁸, Martin Martinot⁷², Pascal Andreu⁷³, Mathieu Blot⁷⁴, Lionel Piroth⁷⁴, Jean Pierre Quenot⁷⁴, Olivier Epaulard⁷⁵, Nicolas Terzi⁷⁵, Karine Faure⁷⁶, Emmanuel Faure⁷⁶, Julien Poissy⁵⁸, Saad Nseir⁵⁸, Florence Ader⁷⁷, Laurent Argaud⁷⁸, Tristan Ferry⁷⁸, Thomas Perpoint⁷⁸, Vincent Piriou⁷⁸, Jean-Christophe Richard⁷⁸, Julien Textoris⁷⁸, Florent Valour⁷⁸, Florent Wallet⁷⁸, André Cabié⁷⁹, Jean-Marie Turmel⁷⁹, Cyrille Chabartier⁸⁰, Rostane Gaci⁸⁰, Céline Robert⁶⁵, Alain Makinson⁸¹, Vincent Moing⁸¹, Kada Klouche⁸², Olivier Hinschberger⁷³, Joy Mootien⁸³, Sébastien Gibot⁷³, François Goehringer⁶⁷, Antoine Kimmoun⁷³, Benjamin Lefevre⁷³, David Boutoille⁸⁴, Emmanuel Canet⁸⁴, Benjamin Gaborit⁸⁴, Paul Turnier⁸⁴, François Raff⁸⁴, Jean Reignier⁸⁴, Johan Courjon⁸⁵, Jean Dellamonica⁸⁵, Sylvie Leroy⁸⁵, Charles-Hugo Marquette⁸⁵, Paul Loubet⁸², Claire Roger⁸², Albert Sotto⁸², Cédric Bruel⁸², Benoît Pilmis⁸⁶, Guillaume Geri⁸⁶, Elisabeth Rouveix-Nordon⁸⁷, Olivier Bouchaud⁸⁸, Samy Figueiredo⁸⁶, Stéphane Jaureguiberry⁸⁶, Xavier Monnet⁸⁹, Lila Bouadma⁸⁶, François-Xavier Lescure⁸⁶, Nathan Peiffer-Smadja⁸⁶, Jean-François Timsit⁸⁶, Yazdan Yazdanpanah⁹⁰, Solen Kerneis⁹¹, Marie Lachâtre⁹¹, Odile Launay⁹², Jean-Paul Mira⁹², Julien Mayaux⁹², Valérie Pourcher⁹¹, Jérôme Aboab⁹³, Flora Crockett⁹⁴, Naomi Sayre⁹⁴, Clément Dubost⁹⁴, Cécile Ficko⁹⁴, David Lebeaux⁹⁵, Sébastien Gallien⁸⁷, Armand Mekontso-Dessap⁹⁶, Jérôme Pavet⁹⁷, François Stefan⁹⁸, Hafid Ait-Oufella⁹⁹, Karine Lacombe⁹⁹, Jean-Michel Molina⁸⁷, Murielle Fartoukh⁶⁴, Gilles Pialoux⁶⁴, Firouzé Bani-Sadr¹⁰⁰, Bruno Mourvillier⁸⁶, François Benezit⁵⁹, Fabrice Laine⁵⁹, Bruno Laviolle⁵⁹, Yves Tulzo⁵⁹, Matthieu Revet⁵⁹, Elisabeth Botelho-Nevers¹⁰¹, Amandine Gagneux-Brunon¹⁰¹, Guillaume Thiery¹⁰¹, François Danion⁷², Yves Hansmann⁷², Ferhat Meziani⁷², Walid Oulehri⁷², Charles Tacquard⁷², Fanny Bounes-Vardon¹⁰², Guillaume Martin-Blondel¹⁰², Marlène Murris-Espin¹⁰², Béatrice Riu-Poulenc¹⁰², Vanessa Jeanmichel¹⁰², Eric Senneville¹⁰³, Louis Bernard¹⁰⁴, Denis Garot¹⁰⁵, Jean Reuter⁷³, Thérèse Staub⁷³, Marc Berna⁷³, Sandra Braz¹⁰⁶, Joao Miguel Ferreira Ribeiro¹⁰⁶, José-Artur Paiva¹⁰⁷, Roberto Roncon-Albuquerque¹⁰⁷ & Benjamin Leveau¹⁰⁸

⁴⁷INSERM, Pôle de Recherche Clinique, Paris, France. ⁴⁸Department of Pulmonary Medicine, Kepler University Hospital, Linz, Austria. ⁴⁹Division of Intensive Care and Emergency Medicine, Medical University of Innsbruck, Innsbruck, Austria. ⁵⁰Cancer Cluster Salzburg, Paracelsus Medical University, Salzburg, Austria. ⁵¹Department of Internal Medicine and Infectious Diseases, Centre Hospitalier Régional (CHR) de Liège, 4000 Liège, Belgium. ⁵²Department of Intensive Care, University Hospital of Liège, Liège, Belgium. ⁵³Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium. ⁵⁴Saint-Luc University Hospital, Brussels, Belgium. ⁵⁵Université de Montréal, Montreal, Canada. ⁵⁶CHU de Grenoble Alpes, Grenoble, France. ⁵⁷Toulouse University Hospital, Toulouse, France. ⁵⁸CHU Lille, Lille, France. ⁵⁹CHU Rennes, Rennes, France. ⁶⁰ANRS-MIE, Paris, France. ⁶¹Centre Hospitalier de Cayenne, Cayenne, French Guiana, France. ⁶²Institut Pasteur de la Guyane, Cayenne, French Guiana, France. ⁶³Amiens University Hospital, Amiens, France. ⁶⁴Hôpital Tenon, AP-HP, Paris, France. ⁶⁵CHU de Bordeaux, Bordeaux, France. ⁶⁶Besançon University Hospital, Besançon, France. ⁶⁷CHU de Besançon, Besançon, France. ⁶⁸CHU Nice, Nice, France. ⁶⁹CHU de La Rochelle, La Rochelle, France. ⁷⁰CHU de Rennes, Rennes, France. ⁷¹INSERM, University of Bordeaux, Talence, France. ⁷²CHU Strasbourg, Strasbourg, France. ⁷³CHU de Nancy, Nancy, France. ⁷⁴CHU Dijon, Dijon, France. ⁷⁵CHU Grenoble Alpes, Grenoble, France. ⁷⁶CHU Saint-Etienne, Saint-Etienne Cedex 2, France. ⁷⁷INSERM, Hospices Civils de Lyon, Lyon, France. ⁷⁸Hospices Civils de Lyon, Lyon, France. ⁷⁹CHU de Martinique, Fort-de-France, France. ⁸⁰CHU de Guadeloupe, Pointe-à-Pitre, France. ⁸¹CHU de Montpellier, Montpellier, France. ⁸²CHU de Nîmes, Nîmes, France. ⁸³CHU de La Réunion, Saint-Denis, France. ⁸⁴CHU de Nantes, Paris, France. ⁸⁵CHU de Nice, Paris, France. ⁸⁶Hôpital Bichat-Claude Bernard, AP-HP, Paris, France. ⁸⁷Hôpital Saint-Louis, AP-HP, Paris, France. ⁸⁸Hôpital Avicenne, AP-HP, Bobigny, France. ⁸⁹Hôpital de Bicêtre, AP-HP, Le Kremlin-Bicêtre, France. ⁹⁰INSERM & Hôpital Bichat-Claude Bernard, Paris, France. ⁹¹Hôpital Pitié-Salpêtrière, AP-HP, Paris, France. ⁹²Hôpital Cochin, AP-HP, Paris, France. ⁹³Hôpital Louis-Mourier, AP-HP, Colombes, France. ⁹⁴AP-HP, Paris, France. ⁹⁵Hôpital Necker-Enfants Malades, AP-HP, Paris, France. ⁹⁶Hôpital Henri-Mondor, AP-HP, Créteil, France. ⁹⁷Hôpital Marie-Lannelongue, Paris Sud, Orsay, France. ⁹⁸CHU, AP-HP, Paris, France. ⁹⁹Hôpital Saint-Antoine, AP-HP, Paris, France. ¹⁰⁰CHU de Reims, Reims Cedex, France. ¹⁰¹CHU Saint-Étienne, Saint-Etienne Cedex 2, France. ¹⁰²CHU de Toulouse, Toulouse, France. ¹⁰³CH Dunkerque & CH Tourcoing, Dunkerque Cedex 1, France. ¹⁰⁴CHU de Tours, Tours, France. ¹⁰⁵Centre Hospitalier Le Mans, Le Mans, France. ¹⁰⁶Centro Hospitalar Universitário de Lisboa Norte, Lisboa, Portugal. ¹⁰⁷Centro Hospitalar Universitário São João, Porto, Portugal. ¹⁰⁸Hôpital de La Croix-Rousse, Centre de Ressources Biologiques Nord, Hospices Civils de Lyon, 26900 Lyon, France.