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Biomarkers trajectories in critically ill patients with COVID-19 acute respiratory distress syndrome: insights from latent class growth analysis

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Abstract

Background Acute respiratory distress syndrome (ARDS) is a common condition requiring intensive care, with limited effective treatments due to its clinical and biological heterogeneity. Efforts in critical care medicine have identified ARDS sub-phenotypes, hyperinflammatory and hypoinflammatory, which suggest underlying heterogeneity in patient responses. Studies have detected a low prevalence of the hyperinflammatory phenotype in ARDS patients related to Coronavirus Disease 2019 (COVID-19). To identify targeted therapeutic interventions, this study applied growth mixture models to longitudinal biomarker data to determine specific latent trajectory groups.

Methods This is a secondary analysis of a cohort study on patients with COVID-19 pneumonia admitted to an Italian intensive care unit (ICU) with at least two assessments of inflammatory marker levels within 28 days of admission. Plasma levels of interleukin 6 (IL-6), interleukin-8 (IL-8), soluble tumour necrosis factor receptor 1 (sTNFR-1), intercellular adhesion molecule 1 (ICAM-1), soluble receptor for advanced glycation end products (sRAGE) and angiopoietin-2 (Ang2) were assessed.

Results Fifty-eight patients were analysed, with a total of 201 level assessments performed. None showed hyperinflammatory phenotype within 48 h of ICU admission. Latent class growth analysis identified distinct trajectories for sTNFR-1, ICAM-1, and sRAGE, with sTNFR-1 class 1 including 39.7% of patients showing elevated levels escalating during ICU stay. Compared to sTNFR-1 class 2, class 1 patients were older (63.8 ± 14.7 versus 58.4 ± 12.7, $P = 0.0486$), had higher baseline inflammatory marker levels (IL-6, IL-8, sRAGE, and sTNFR-1), higher proportions of prone positioning and continuous renal replacement therapy utilization, and a higher mortality rate (56.5% versus 28.6%, $p = 0.0333$).

Conclusions In our cohort of critically ill patients with severe COVID-19, we identified distinct latent trajectories based on longitudinal data of inflammatory markers. sTNFR-1 levels identified a subgroup with a hypoinflammatory

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phenotype, demonstrating a mortality rate comparable to that typically observed in ARDS hyperinflammatory phenotype. These findings point out the criticality of delineating distinct patient subgroups within the context of ARDS and COVID-19, enhancing clinical management and optimizing patient prognosis.

Keywords ARDS, Latent class growth analysis, Phenotyping

Background

Acute respiratory distress syndrome (ARDS) is a common condition in patients admitted to intensive care unit (ICU) that often requires respiratory support with invasive mechanical ventilation. Since ARDS is characterized by considerable clinical and biological heterogeneity, in the last decade significant efforts have been made to identify subphenotypes of the syndrome based on genomics, transcriptomics, proteomics, and metabolomics with the aim of developing personalized diagnostic and therapeutic approaches. Algorithms based on clinical variables and biomarker levels allowed the identification of two distinct ARDS subphenotypes (hyper- and hypoinflammatory) with different clinical outcome and responses to treatment[1, 2].

The hyperinflammatory subphenotype is characterized by severe inflammation (increased plasma levels of inflammatory biomarkers), shock, metabolic acidosis, and poorer patient outcome. In contrast, patients with the hypoinflammatory form of ARDS have milder inflammatory response and higher survival rates. Studies performed during the recent pandemic have demonstrated that in patients with Coronavirus Disease 2019 (COVID-19)-related ARDS, the hyperinflammatory subphenotype is less prevalent compared to non-COVID-19 ARDS cases [2]. In a recent study involving 400 cases with severe COVID-19, the typical hyperinflammatory form of ARDS was identified only in one patient, while analysis of baseline biomarkers and clinical variables allowed the identification of two novel latent classes named subtype 1 and subtype 2 [3]. Subtype 2, comprising 27% of patients, showed persistent inflammation, epithelial, and endothelial injury, with a doubled 28-day mortality rate compared to subtype 1 (41% versus 20%). Importantly, algorithms for defining ARDS subphenotypes rely only on baseline data and may not capture the evolving nature of the disease, potentially leading to shifts in phenotypes over time that could impact on clinical outcome [3].

In a large multicentre study on critically ill patients with COVID-19-related ARDS, we analysed the trajectories of clinical, ventilatory, and laboratory parameters throughout the ICU stay, revealing significant associations between daily values, trends, and patient outcomes [4]. Bos LDJ et al. identified distinct subgroups of COVID-19 patients by analysing the trajectory of respiratory compliance, mechanical power, PaO_2/FiO_2, and ventilatory ratio during the initial 96 h following ICU admission [5]. Finally, in patients with moderate-severe ARDS, a recent study demonstrated changes in the expression of individual genes within the first two days of enrolment in both hypo- and hyperinflammatory subphenotypes [6].

In the present study, we aimed to: (a) confirm the prevalence estimates of COVID-19-related ARDS phenotypes; (b) explore whether specific latent groups of trajectories are identifiable within each phenotype by applying growth mixture models for uncovering latent evolutions in the longitudinal data of various biomarkers.

Methods

Study design

This is a secondary analysis of a multicentre cohort study that enrolled all consecutive patients with COVID-19 admitted to 27 Italian ICUs during 2020–2022. The study, entitled "Epidemiological and Clinical Characteristics of Critically Ill Patients With COVID-19" (2019nCoV-ICU), was conducted in accordance with the Declaration of Helsinki and received approval from the Institutional Review Board; it was registered on clinicaltrials.gov (Clinical Trial Number: NCT04388670; Registration Date: 2020-05–12). Written informed consent was deferred or waived as per local regulations. The study details have been previously published [4, 7–9].

Patients

In this analysis, the study population consisted of patients with COVID-19 pneumonia admitted to a single ICU (IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico, located in Milan), with at least two separate evaluations obtained on different ICU days within 28 days from ICU admission of specific plasma biomarkers related to inflammation, endothelial activation, and epithelial injury, with the initial evaluation occurring within 7 days of ICU stay (Figure S1).

Data collection

Clinical patient data were recorded daily on an electronic database (REDCap, Research Electronic Data Capture; Vanderbilt University, Nashville, TN, USA) by an experienced intensivist. Demographic data (age, sex, pre-existing comorbidities, chronic medications, date of symptoms onset) were recorded at ICU admission.

Inflammation markers levels quantification

Blood samples were collected from patients admitted to ICU at multiple time points during the ICU stay, specifically within the first 48 h of admission, and on the 3rd,

7th, and 14th day after ICU admission. Due to issues related to the hospital's healthcare organizational aspect during the COVID-19 pandemic, efforts were made to adhere to these timelines, but it was not always feasible. Plasma, obtained by centrifugation within 1 h from sampling, was stored at −80 °C until analyses were performed using a custom magnetic microparticle-based 6-plex human cytokine panel (R&D Systems, Minneapolis, USA) and LABScanTM 100 flow cytometer (Luminex Corporation, Austin, TX, USA). The following analytes were evaluated: interleukin 6 (IL-6), interleukin-8 (IL-8), soluble tumour necrosis factor receptor 1 (sTNFR-1), intercellular adhesion molecule 1 (ICAM-1), soluble receptor for advanced glycation end products (sRAGE) and angiopoietin-2 (Ang2). The assays were strictly performed according to the manufacturer's protocols and recommendations. Cytokine levels were expressed in pg/ml. Reference standards were assayed in duplicate and standard curves extended down by one dilution point. Data were acquired and analysed with xPONENT software (ver. 4.2, Luminex Corporation, Austin, TX, USA) with a detection target of 50 beads per region and a recommended doublet discriminator (DD) gates of 8,000–16,500.

Statistical analysis

Continuous variables were reported as mean and standard deviation (SD) or median and interquartile range (IQR), depending on data distribution. Discrete variables were expressed as absolute and relative frequencies, and normality was assessed using the Shapiro-Wilk test.

The probabilities of phenotype assignment were estimated using the three-variable parsimonious classifier model proposed by Sinha et at., based on values from the first 48 h after ICU admission [2]:

Probability (hyperinflammatory phenotype) = -11.9593 + 1.0138 × ln(IL-6+1) - 0.2436 × HCO_3^- + 1.1913 × ln (sTNFR-1+1)

Here, IL-6 represents interleukin 6 (pg/mL), HCO_3^- is serum bicarbonate (mmol/L), and sTNFR-1 corresponds to soluble tumour necrosis factor receptor 1 (pg/mL).

Patients whose predicted probability was 0.5 or higher were categorized as having a hyperinflammatory phenotype; otherwise, they were classified as having a hypoinflammatory phenotype. An alternative cut-off of 0.274 was employed, in line with the Youden's index from Sinha's development model. To assess the changes in the prevalence of phenotypes during ICU stay, probabilities of hyperinflammatory phenotype was analogously predicted for each day when the three variables (IL-6, HCO_3^-, sTNFR-1) were available.

Latent Class Growth Analysis (LCGA) was used to identify distinct biomarker trajectory patterns (classes) over time, without a priori knowledge of grouping variables [10, 11]. Due to the potential convergence issues in the growth mixture models caused by skewed biomarker data, we addressed this by applying a Box-Cox transformation to mitigate skewness and to guarantee the normal distribution of model residuals [12]. Model optimization was performed with 100 random starts, and the optimal number of latent trajectories (two or three-class solutions) was determined using goodness-of-fit criteria (Akaike's Information Criterion (AIC), Bayesian Information Criterion (BIC), and sample size-adjusted BIC (SABIC)) recommended in the literature [10]. Classification quality was assessed using entropy, with models containing fewer than 10 patients per class excluded. In an effort to improve the model specification and better capture longitudinal variability, we tested growth mixture models (GMMs) including random intercepts and slopes to account for within-class heterogeneity in biomarker trajectories (i.e., to allow patients belonging to the same latent class to have individualized starting values and rates of change over time, rather than assuming identical progression within each class).

Finally, after the best fitting latent trajectory model was identified, the predicted posterior probabilities of belonging to each class were used for allocating patients to their most likely class. Once classes were derived, class membership was treated as observed variable and its relationship with earlier explanatory variables (such as age, sex, BMI, time from COVID-19 symptoms, comorbidities, Charlson comorbidity index, SOFA score, clinical data, and biomarker concentrations at ICU admission) was assessed through appropriate statistical tests. Mixed-effects models with random intercepts and slopes were applied to assess the effect of class membership and low-dose steroid use on individual biomarker trajectories over time.

No imputation of missing data was performed for the purposes of the analysis. A significance α-level of 0.05 was used for all statistical tests. Analyses were performed using R 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria) and SAS 9.4 (SAS Institute, Cary, North Carolina, USA).

Further methodological details are provided in the Supplementary Material.

Results

We included in the analysis 58 patients admitted to ICU due to COVID-19 pneumonia (Figure S1) between April 2020 and December 2021. 50% of the patients had at least 3 (IQR 3–4) assessments of inflammatory biomarkers during the 28 days following ICU admission, for a total of 201 quantifications performed by the laboratory (Fig. 1, panel A-F). The first sample was collected within 24 h for 32 patients (55.2%), between days 2 and 5 for 14 patients (24.1%), and between days 6 and 7 for 12 patients

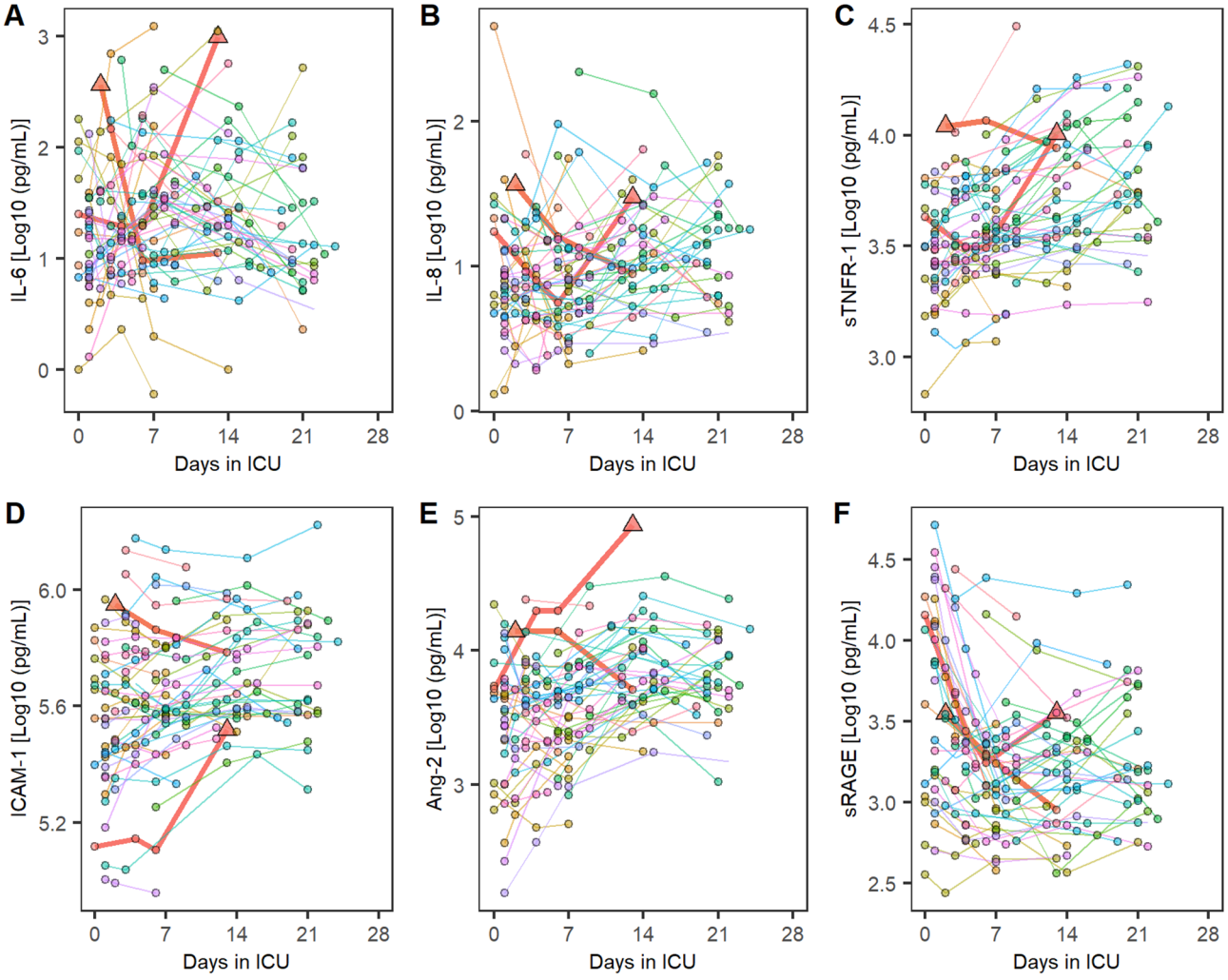

Fig. 1 Longitudinal trends of inflammatory biomarkers levels over 28 days following ICU admission. The figure reports individual patient trajectories of **i**nterleukin 6 (IL-6) levels (Panel **A**), interleukin 8 (IL-8) levels (Panel **B**), soluble tumour necrosis factor receptor 1 (sTNFR-1) levels (Panel **C**), intercellular adhesion molecule 1 (ICAM-1) levels (Panel **D**), angiopoietin-2 (Ang-2) levels (Panel **E**), and soluble receptor for advanced glycation end products (sRAGE) levels (Panel **F**) measured repeatedly over a 28-day period following ICU admission. Biomarker values are plotted on a logarithmic (base-10) scale to facilitate visualization of dynamic changes. Each coloured line represents the trajectory of a single patient. Solid lines represent trend for patients who presented a hyperinflammatory phenotype during ICU stay. The timepoint at which the hyperinflammatory phenotype was identified is marked with a triangle

(20.7%). The cumulative distribution of the day of collection of plasma levels of inflammatory markers is reported in Figure S2. To further characterize sampling across ICU time intervals, Figure S3 shows the number of valid biomarker quantifications per week, confirming that all samples included complete measurement of the six markers under study, except for a single sample (0.05%) in which IL-6 and IL-8 could not be quantified.

The study population predominantly comprised males (69%), and the mean age was 60.6 years (SD 13.7). Half of the patients were admitted to the ICU within 12 days from the onset of COVID-19 symptoms. The median Charlson comorbidity index at ICU admission was 3 (IQR 1–4). Among the prevalent chronic conditions, diabetes was the most common, affecting 29.3% of the participants. At baseline, 86% of patients were mechanically ventilated. Relevant clinical parameters at baseline (within two days from admission) are reported in Table 1. Notably, during ICU stay (median duration of 24 days, IQR 14–44), 39 patients (69.6%) received low-dose steroids (6 mg of dexamethasone daily, as per the RECOVERY trial), and of these, 74.4% started steroid therapy before the first cytokine assessment and no statistical differences in first cytokine assessment were observed between those who started treatment before or after the assessment (Table S1). Additionally, there were no significant differences in biomarker levels at ICU admission between patients treated with low-dose steroids and those who were not, with the exception of Ang-2 ($p = 0.0237$), for which no different trajectory over time

Table 1 Characteristics of the study population at ICU admission and outcomes

	Total population
Patients (n)	58
Onset period of COVID-19, n (%)	
Apr 2020 – Sept 2020	11 (18.97)
Oct 2020 – Dec 2020	11 (18.97)
Jan 2021– Dec 2021	36 (62.07)
Sex, n (%)	
Male	40 (68.97)
Female	18 (31.03)
Comorbidities, n (%)	
Cardiovascular disease	7 (12.07)
Pulmonary disease	7 (12.07)
Liver disease	4 (6.90)
Diabetes	17 (29.31)
Malignancy	7 (12.07)
Immunological disease	9 (15.52)
Age (years), mean ± SD	60.55 ± 13.67
BMI (kg/m^2), mean ± SD	30.21 ± 7.31
Time for symptoms (days), median [IQR]	12 [8–17]
Charlson comorbidity index, median [IQR]	3 [1–4]
Clinical parameters at baseline[a]	
SOFA score, median [IQR]	4 [3–5]
Invasive mechanical ventilation, n (%)	50 (86.21)
Respiratory rate (breaths/min), mean ± SD	20.41 ± 5.51
TV (ml/kg), mean ± SD	7.24 ± 1.29
FiO_2 (%), median [IQR]	70 [60–90]
PEEP (cmH_2O), mean ± SD	11.13 ± 2.52
Minute ventilation (l/min), mean ± SD	8.82 ± 2.28
pH, mean ± SD	7.38 ± 0.09
HCO_3^- (mmol/l), mean ± SD	27.23 ± 5.34
$PaCO_2$ (mmHg), mean ± SD	47.20 ± 12.11
PaO_2 (mmHg), mean ± SD	83.88 ± 23.30
PaO_2/FiO_2 (mmHg), median [IQR]	113 [84–144]
Lactate (mmol/l), median [IQR]	1.30 [0.95–1.60]
Bilirubin (mg/dl), median [IQR]	0.57 [0.41–0.79]
C-reactive protein (mg/L), mean ± SD	11.39 ± 9.74
Ferritin (µg/L), median [IQR]	1,026 [631-2,447]
D-Dimer (ng/mL), median [IQR]	1,855[695-5,065]
Inflammation markers levels at baseline (n = 33)[a]	
IL-6 (pg/mL), median [IQR]	16.75 [6.80–35.20]
IL-8 (pg/mL), median [IQR]	7.20 [4.60–15.10]
Ang-2 (pg/mL), median [IQR]	3,557 [1,012 – 5,567]
sRAGE (pg/mL), median [IQR]	4,821 [1,133 – 13,719]
ICAM-1 (pg/mL), median [IQR]	359,166 [236,928 – 502,351]
sTNFR-1 (pg/mL), median [IQR]	2,881 [2,130-4,135]
Treatment in ICU, n (%)	
Steroids (low dose) [b]	39 (69.64)
Anti-interleukins drugs[c]	7 (12.50)
Prone positioning	39 (69.64)
CRRT	14 (25.00)
ECLS	3 (5.36)
Outcomes	

Table 1 (continued)

	Total population
Duration of ventilation (days), median [IQR]	17 [7–31]
ICU mortality, n (%)	23 (39.66)

Abbreviations: Ang-2, Angiopoietin-2, *BMI*, body mass index, *CRRT*, continuous renal replacement therapy, *ECLS*, extracorporeal life support, FiO_2, fraction of inspired oxygen, HCO_3, serum bicarbonate, *ICAM-1*, intercellular adhesion molecule 1, *ICU*, intensive care unit, *IL-6*, interleukin 6, *IL-8*, interleukin 8, *IQR*, interquartile range [1st quartile-3rd quartile], $PaCO_2$, partial pressure of carbon dioxide, PaO_2, partial pressure of oxygen, *PEEP*, positive end-expiratory pressure, *SD*, standard deviation, *SOFA*, Sequential (Sepsis-Related) Organ Failure Assessment, sRAGE, soluble receptor for advanced glycation end products, *sTNFR-1*, soluble tumour necrosis factor receptor 1, *TV*, tidal volume

[a] First measurement within 2 days from *ICU* admission

[b] Treatment was initiated prior to the first biomarker assessment in 29 patients (74.4%)

[c] Anti-interleukins drugs include anakinra (4 patients) and tocilizumab (3 patients)

was observed based on treatment (Table S2, Figure S4). Furthermore, a significant trend over time was observed for ICAM-1, sRAGE, and sTNFR-1, but this was not associated by low-dose steroid use, nor did their temporal evolution differ between treatment groups (Figure S4).

Seven patients (12.5%) received anti-interleukin drugs therapy, with treatment initiation occurring after the first cytokine assessment in all cases and no significant differences in cytokine levels were observed at ICU admission between those treated or not with anti-interleukin drugs, except for sTNFR-1 ($p = 0.0486$) (Table 1, Table S2).

Appling the three-variable parsimonious classifier model and considering a threshold of 0.5 in the predicted probabilities, hyperinflammatory phenotype was never detected during the ICU stay (Figure S5). Decreasing the cut-off to 0.274, only two patients showed an hyperinflammatory phenotype during the ICU stay in only two different time points, but not during the first 48 hours.

Latent class growth analysis

Based on goodness-of-fit criteria and entropy values, LCGA indicated a minimum of two distinct trajectories for sTNFR-1, ICAM-1 and sRAGE (Table S3), while the analysis did not identify acceptable solutions for IL-6, IL-8, and Ang-2 as significantly entropy did not reach the specified threshold of 0.8. For IL-6, a model with three latent trajectories provided an entropy level of 0.85 but was excluded due to one trajectory class including only one patient. Attempts to apply GMM to the same biomarkers did not yield robust solutions: entropy values were consistently lower compared to LCGA, and convergence was unstable (Table S3). The predicted trajectories are showed in Fig. 2 and fixed effects in the longitudinal models are reported in Table S4. For sTNFR-1, class 1 identified a subgroup of patients (39.66%) characterized by elevated levels at ICU admission that rapidly escalated during their ICU stay (Table S5 and Fig. 2, Panel A). The second class included patients (60.34%) characterized by a trajectory with lower sTNFR-1 levels, demonstrating an increase during ICU stay. If we consider the presence of these two latent classes within the study population, individuals in sTNFR-1 class 1 were statistically significantly older (mean ± SD: 63.83 ± 14.74 age vs. 58.40 ± 12.68, $p = 0.0486$). Moreover, patients in the same class had a higher Charlson Comorbidity Index and higher levels of IL-6, IL-8, sRAGE, and sTNFR-1 observed at baseline. Furthermore, sTNFR-1 class 1 was associated with more frequent use of prone positioning (85.71% vs. 60.00%, $p = 0.0428$) and of continuous renal replacement therapy (CRRT) (42.86% vs. 14.29%, $p = 0.0168$) and with higher mortality rate (56.52% vs. 28.57%, $p = 0.0333$) (Table S5, Fig. 3). Based on sTNFR-1 classes, Fig. 4 shows the estimated linear trends of each biomarker of interest (IL-6, IL-8, ICAM-1, Ang-2, sRAGE) during the ICU stay. With the exception of ICAM-1, biomarkers exhibited variations between classes at all time points, with a significant positive trend observed for Ang-2. Regarding ICAM-1, a significant positive trend was identified in both classes, although the values did not differ significantly between the groups.

Two latent classes with incremental trajectories over time were identified for ICAM-1 longitudinal levels and no differences between these classes were detected in terms of baseline characteristics (excluding pH) or clinical outcomes during the ICU stay (Fig. 2, Panel B, Table S6, and Fig. 3).

A three-class LCGA model was chosen as the most suitable for sRAGE: the first class (25.86% of the population) consisted of subjects with the lowest sRAGE levels at baseline, remaining stable throughout their ICU stay; the second class (17.24%) included subjects with medium baseline levels that increased over time, while the third class (56.90%) encompassed subjects with the highest baseline levels that decreased over time (Fig. 2, Panel C). Patients classified in sRAGE class 1 had lower baseline levels of C-reactive protein, shorter time from COVID-19 symptoms onset to ICU admission, and only one patient underwent CRRT during ICU stay (Table S7). Moreover, these patients showed an sRAGE trajectory characterized by the lowest levels of the biomarker during their ICU stay, with treatments and outcomes that did not differ between the three identified classes (Table S7 and Fig. 3).

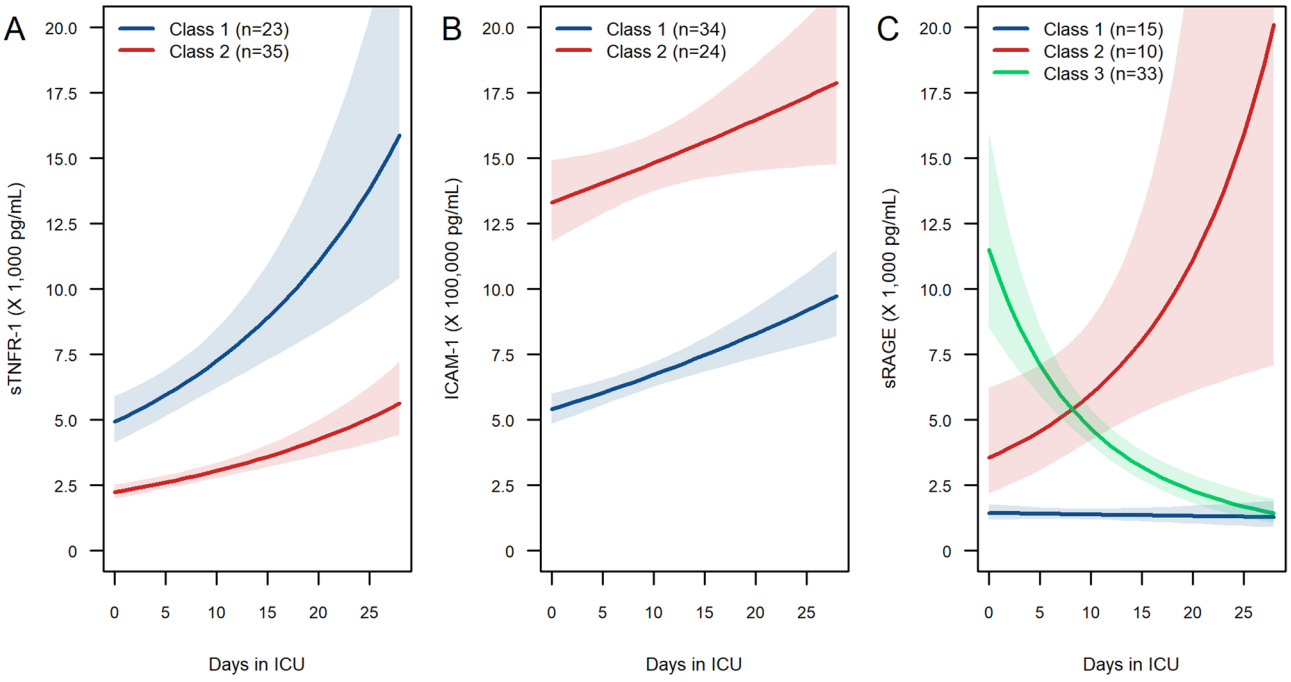

Fig. 2 Predicted trajectories for markers of inflammation (sTNFR-1, ICAM-1, sRAGE), using LCGA. Panel (**A**) The longitudinal data of sTNFR-1 identified two latent classes of patients, with 39.7% classified in class 1 and 60.3% in class 2. Panel (**B**) The longitudinal data of ICAM-1 identified two latent classes of patients, with 58.6% classified in class 1 and 41.4% in class 2. Panel (**C**) The longitudinal data of sRAGE identified three latent classes of patients, with 25.9% classified in class 1, 17.2% in class 2 and 69.7% in class 3

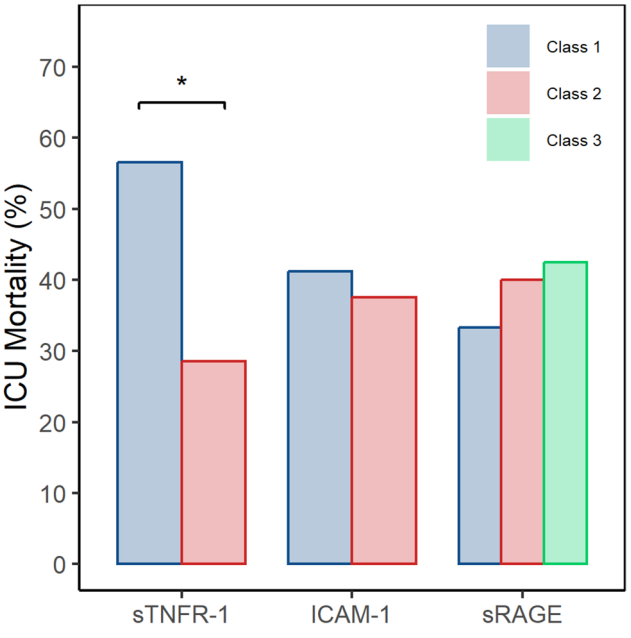

Fig. 3 ICU mortality in patient subgroups defined by sTNFR-1, ICAM-1, and sRAGE levels using LCGA. ICU mortality rates vary significantly across the sTNFR-1 subgroups ($p = 0.0333$), also after adjusting for age and Charlson Comorbidity Index ($p = 0.0039$)

No class, as defined by each biomarker trajectory, showed a different prevalence of patients treated with low-dose steroids (Table S5 - S7).

Discussion

In this work, we investigated subphenotypes of ARDS in critically ill patients with COVID-19 using the model proposed by Sinha P. et al. and we observed that the hyperinflammatory phenotype was never detectable at ICU admission.[2] After 48 h, only two patients showed a hyperinflammatory phenotype using a threshold of 0.274 for the discrimination between phenotypes [2].

To further explore the inflammatory landscape, we applied an exploratory LCGA approach to analyse the trajectories of specific biomarkers of inflammation, epithelial and endothelial damage during the ICU stay. The advantage of this approach is that LCGA is a person-centred method that probabilistically assigns subjects into latent classes based on similar patterns of longitudinal data, without defining a patient solely from their clinical outcomes. At least two classes of patients with distinct trajectories were identified for sTNFR-1, ICAM-1, and sRAGE, indicating potential differences in the underlying pathophysiological processes. Conversely, no statistically acceptable solutions were detected for IL-6, IL-8, and Ang-2 trajectories.

Our analysis also focused on the relationship between these biomarkers' trajectories and clinical outcomes,

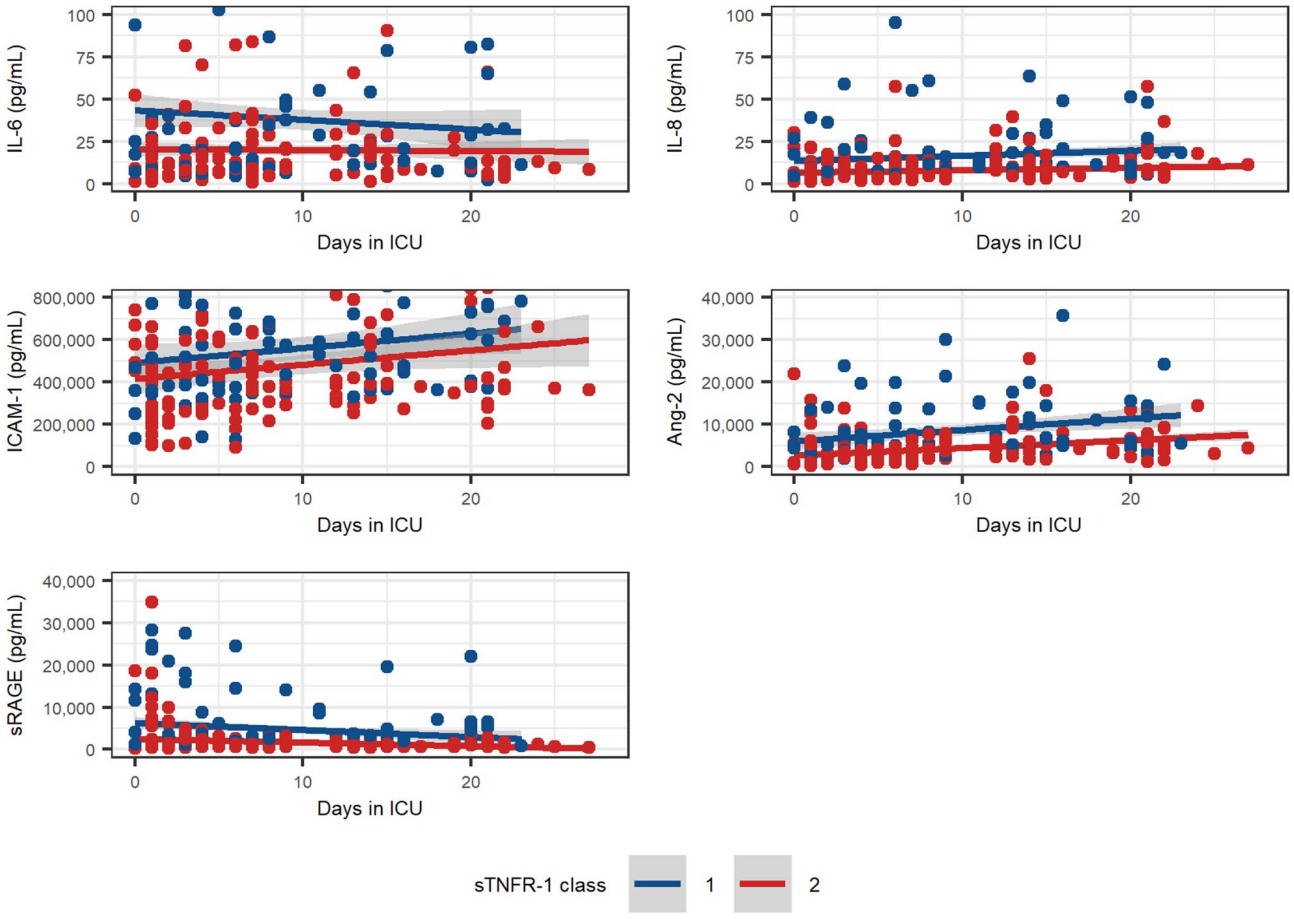

Fig. 4 Longitudinal analysis of biomarker variations over time, stratified by sTNFR-1 classes. Mixed-effects models for longitudinal repeated measures were applied to assess the association between biomarkers and time, stratified by sTNFR-1 classes. The IL-6 model identified a significant effect of the class ($p=0.0085$), with no effect of time ($p=0.3265$) or interaction ($p=0.4490$). Similarly, the IL-8 model showed a significant class effect ($p=0.0023$), but no effect of time ($p=0.2318$) or interaction ($p=0.6405$). In contrast, the ICAM-1 model revealed a significant effect of time ($p=0.0137$), with no effect of class ($p=0.1680$) or interaction ($p=0.6985$). The Ang-2 model demonstrated both significant time ($p=0.0013$) and class effects ($p=0.0008$), with no significant interaction ($p=0.7429$). The sRAGE model showed no significant time effect ($p=0.1806$) or interaction ($p=0.3645$), and no significant class effect ($p=0.1806$)

particularly ICU mortality. ICU mortality rates were significantly different only between two classes defined by the trajectory of sTNFR-1 plasma levels. Patients classified under sTNFR-1 class 1 had significantly higher mortality rates compared to class 2 patients (56.5% versus 38.6%). Moreover, class 1 patients were older, had more comorbidities, higher baseline plasma levels of IL-8 and sRAGE and increased utilization of prone positioning and CRRT, but no difference in low-dose steroid therapy.

Mortality rate in class 1 exceeded 50%, similar to that observed in COVID-19 ARDS patients with the hyperinflammatory phenotype and higher than that observed in COVID-19 patients identified as "subtype 2" according to the model recently proposed by Alipanah-Lechner et al. [2, 3].

Our findings highlight the utility of sTNFR-1 as a potential biomarker for risk stratification and prognostication in critically ill COVID-19 patients, assisting in the identification of subjects at higher risk for adverse outcomes who may need tailored therapeutic interventions. sTNFR-1, a soluble form of tumour necrosis factor receptor 1, plays a pivotal role in modulating the actions of TNFα, a key cytokine involved in inflammation and sepsis. It exerts its influence by binding to circulating TNFα molecules, thereby attenuating the inflammatory response [13, 14]. In addition, sTNFR-1 reduces the availability of TNFR-1 on cell surfaces, further regulating TNFα activity [15]. Elevated levels of sTNFR-1 have been associated with increased levels of other inflammatory biomarkers, with higher clinical severity, and an increased risk of developing acute kidney injury and they have been identified as an independent predictor of 30-day mortality [16, 17].

These findings suggest that the increased mortality in COVID-19-associated ARDS may not solely result from the upregulation of the inflammatory pathway,

highlighting the importance of longitudinal biomarkers trajectories to identify potential patient subgroups and improve our understanding of the clinical and biological heterogeneity of ARDS [3, 5, 18]. The simple evaluation of baseline biological characteristics provides only a limited snapshot and does not capture the complexity of the dynamic response to the disease and to its treatment. Indeed, at baseline, no differences were found between the two sTNRF-1 classes in terms of "classical" ARDS sub-phenotyping (all patients showed hypoinflammatory phenotype) and of disease severity, based on clinical (PaO_2/FiO_2, $PaCO_2$, or $HCO3^-$), ventilatory (PEEP, RR, or FiO_2) and inflammatory (CRP or ferritin) parameters [19]. Notably, key markers such as IL-6 and ICAM-1, crucial in discriminating between hyper- and hypo-inflammatory phenotypes in ARDS, did not differ between sTNFR-1 classes. This is in line with recent studies, including those performed in patients with severe COVID-19, highlighting the complex nature of inflammatory responses in such critical conditions [3].

When assessing the biomarkers trajectory in the two sTNFR-1 classes, we observed persistent differences in the average levels of markers of inflammation, endothelial, and epithelial injury, except for ICAM-1 levels. Although the average biomarker levels differ between classes, the way these levels change over time appears to be quite similar across groups. In detail, IL-6 and IL-8 levels remained statistically highest in class 1, possibly due the continuous increase in sTNFR-1 levels, which may have triggered their synthesis [20, 21].

In our study, the two classes of sTNFR-1 showed different levels of epithelial injury biomarker (sRAGE) and endothelial injury biomarker (Ang-2) at baseline and remained elevated over time without significant differences in their trajectories between classes. Similarly, Jabaudon et al. [22] demonstrated that sRAGE levels are elevated in patients with ARDS, with or without sepsis, compared to those solely presenting with sepsis but not ARDS. Moreover, they identified an association between sRAGE levels and the severity of lung injury. Additionally, a positive correlation between plasma SARS-CoV-2 load and sRAGE concentrations has been observed [23]. Particularly noteworthy is the strong association between sRAGE levels and plasma SARS-CoV-2 RNA concentrations, as well as viral kinetics [23].

Additionally, Ang-2 levels were higher at baseline in sTNFR-1 class 1, but their changes over time were similar across classes. Although both sRAGE and Ang-2 serve as indicators of lung tissue damage, they identify different types of injury: sRAGE is linked to acute alveolar epithelial damage, whereas Ang-2 is associated with endothelial injury. Controversial results have been published about the pathophysiology of lung damage in COVID-19, with some studies indicating a predominance of pulmonary endothelial injury and others showing a predominant alveolar epithelial injury [23, 24]. Importantly, although Ang-2 levels differed at baseline between sTNFR-1 trajectory classes, the proportion of patients receiving low-dose steroids was similar across groups, suggesting that treatment alone does not explain these differences. Aside from Ang-2, whose levels differed at ICU admission between treated and untreated patients, no biomarker showed significant variation based on steroid use, and their longitudinal trends remained comparable. These findings are partially consistent with those of de Brabander et al., who reported significant temporal changes in several plasma biomarkers, including CCL20, CXCL8, and CCL2, which varied with corticosteroid exposure [25]. In our cohort, several biomarkers exhibited dynamic temporal patterns, but none were modulated by steroid use. Direct comparison remains limited due to differences in biomarker panels, although both studies investigated inflammatory and endothelial mediators.

A small percentage of patients were treated with anti-interleukin drugs, exclusively initiated after the first cytokine assessment, which might influence cytokine response over time but not directly affect epithelial and endothelial damage biomarkers. Furthermore, trials on COVID-19-associated ARDS using similar anti-inflammatory drugs, such as steroids and anti-interleukin agents, have yielded controversial results [9, 26]. This variability is likely due to the heterogeneous biological response to ARDS and the different management strategies for critically ill patients. Nonetheless, these trials suggest that successful ARDS therapies may be identified by selecting patient subgroups who are more likely to respond to a specific treatment and those at higher risk of poor outcomes. Therefore, our study lays the groundwork for future ARDS research, not only in COVID-19 disease.

Our study has a number of limitations. First, the panel of biomarkers was preselected based on data from non-COVID ARDS and on initial reports in COVID-19. Consequently, we may have overlooked potentially informative biomarkers not included in our panel. Second, despite the planned sampling strategy of samples collection at predefined precise time points, the hospital overload due to the pandemic did not allow us to be consistent with the timeframe. Third, we do not have data regarding the specific strain of SARS-CoV-2, and we cannot exclude that different viral variants could lead to different results. Furthermore, data regarding COVID-19 vaccination (vaccine type and date of vaccination) was not collected.

Although our study was conducted in a single-centre cohort of COVID-19-related ARDS patients, investigating biomarker trajectories in this context remains highly relevant. COVID-19 ARDS shows a distinct

immunopathology, characterized by viral-driven inflammation and endothelial injury, which differentiates it from classical ARDS and supports its use as a model for infectious forms of ARDS [27, 28]. Furthermore, the longitudinal, patient-centred methodology used in our analysis may be applied to other ARDS subtypes, to better understand dynamic pathophysiological mechanisms and individual variability in disease progression and treatment response. Recent evidence suggests that transitions between inflammatory phenotypes over time may carry prognostic significance in critical illness, further supporting the relevance of temporal biomarker profiling in ARDS populations [29].

Concerning methodological limitations, our LCGA lacks the incorporation of random effects to account for individual variations within each latent class, thus assuming an absence of within-class variability in biomarker trajectories. Attempts to introduce random effects into the model, employing a growth mixture model, were hindered by the limited number of observations, resulting in parameter escalation and convergence issues. This challenge is intimately linked to the size of the study population and the number of observations per patient. Consequently, we adopted a fixed-effects LCGA specification, which remained stable and interpretable for hypothesis-generating purposes in this exploratory context. Determining an appropriate sample size for the Latent Growth models remains a nuanced topic in research literature [30, 31]. While literature uniformly acknowledges that this approach may necessitate larger sample sizes compared to other statistical methods, consensus on the required number of observations for a robust study is lacking (e.g., 1,000 subjects, 200 observations, and at least 5 time-points). Thus, it is possible that our sample size is inadequate for precise parameter estimation and that the sample may not be representative, potentially limiting the generalizability of our findings. Moreover, the reduced sample size limited our ability to employ a more advanced approach such as group-based multi-trajectory modelling, wherein latent clusters of individuals with similar trajectories across multiple biomarkers of a target outcome can be identified [32].

These limitations highlight the exploratory nature of our findings, which should be interpreted as hypothesis-generating. Despite the restricted sample size and limited frequency of biomarker assessments, the use of LCGA supported the identification of preliminary patterns in inflammatory trajectories. Notably, recent evidence suggests that temporal transitions between inflammatory phenotypes may carry prognostic significance in critical illness, further supporting the relevance of longitudinal biomarker profiling-even in exploratory settings [29].

However, despite these limitations, our analysis of biomarker trajectories suggested patterns consistent with the dynamic nature of severe ARDS, potentially reflecting the pathophysiology of the disease. In light of the study limitations and the exploratory nature of our findings, we refrained from developing a classifier model aimed at pragmatically identifying subtypes based on the identified sTNFR-1 classes, acknowledging that such a tool would require further testing before any potential research or clinical application. Our observations aim to stimulate further research into the longitudinal dynamics of inflammation in ARDS and the potential prognostic utility of markers such as sTNFR-1. Prospective studies with larger, more diverse cohorts and more frequent biomarker assessments will be essential to validate and extend our findings toward clinical translation.

Conclusions

In our study involving adult ICU patients with severe COVID-19, we identified two latent classes based on sTNFR-1 trajectories, which appeared to be associated with distinct clinical outcomes. Class 1, constituting 39.66% of the cohort, demonstrated persistent inflammation and notable disparities in epithelial and endothelial injury biomarkers. This subgroup experienced double the ICU mortality rate and necessitated three times more CRRT compared to Class 2. These findings, derived from an exploratory LCGA approach applied to both clinical and protein biomarker data, should be considered hypothesis-generating. While this approach may represents a step towards a clinically useful subclassification of ARDS patients, potentially uncovering biological mechanisms that shape clinical outcomes and identify subgroups responsive to target treatments, further validation in larger ICU cohorts is essential, including longitudinal assessment of biomarker trajectories to confirm the relevance and reproducibility of the identified profiles.

Abbreviations

AIC	Akaike's information criterion
Ang2	Angiopoietin-2
ARDS	Acute respiratory distress syndrome
BIC	Bayesian information criterion
COVID-19	Coronavirus disease 2019
CRRT	Continuous renal replacement therapy
HCO_3^-	Bicarbonate
ICAM-1	Intercellular adhesion molecule 1
ICU	Intensive care unit
IL-6	Interleukin-6
IL-8	Interleukin-8
IQR	Interquartile range (first-third quartile)
LCGA	Latent class growth analysis
SABIC	Sample size-adjusted BIC
SD	Standard deviation
SOFA	Sequential organ failure assessment
sRAGE	Soluble receptor for advanced glycation end products
sTNFR-1	Soluble tumour necrosis factor receptor 1

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12890-025-03961-x.

Supplementary Material 1.

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Authors' contributions
FM: formal analysis, methodology, visualization, interpretation of data, writing – original draft; FF: Interpretation of data, writing – original draft; GF: data validation, interpretation of data, writing – original draft; AG: data validation, data curation; EC: Acquisition and data validation, data curation; CSC: supervision to methodology, interpretation of data, writing – review & editing; KD: supervision to methodology, interpretation of data, writing – review & editing; VS: interpretation of data, writing – review & editing; MP: interpretation of data, writing – review & editing; CFF: data collection and validation; MT: data collection and validation; ET: data collection and validation; AZ: conceptualization, supervision, interpretation of data, writing – review & editing; GG: Conceptualization, funding acquisition, project administration, interpretation of data, writing – review & editing. All authors read and approved the final version of the manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The 2019nCoV-ICU study was conducted in accordance with the Declaration of Helsinki and received approval from the Ethical Committee of the promoting centre – Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milano), Comitato Etico Milano Area 2 (protocol no. 0008489; approval date: March 20, 2020). The other participating institutions, together with their respective Ethical Committees that approved the study, are listed in the Supplementary Material. The study was registered on ClinicalTrails.gov (Clinical Trial Number: NCT04388670; Registration Date: 2020-05-12). Written informed consent was deferred or waived as per local regulations.

Consent for publication
Not applicable.

Competing interests
GG received grants and research supports from Fischer&Paykel, MSD, Pfizer. GG participated in a company sponsored speaker's bureau: Getinge, Draeger, Fischer&Paykel, Mundipharma, Viatris. CC receives research funding from the NIH, FDA, DOD, Roche-Genentech, and Quantum Leap Healthcare Collaborative. Additionally, she provides consulting services to Janssen, Vasomune, Gen1e Life Sciences, NGMBio, Calcimedica, Arrowhead, EnliTISA, Novartis, Quark Pharmaceuticals, and Cellenkos. She is also a co-recipient of a patent and serves as a council member of the International Sepsis Forum. The remaining authors have disclosed that they do not have any potential conflicts of interest.

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