

# Markers of endothelial and epithelial pulmonary injury in mechanically ventilated COVID-19 ICU patients

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**Research**

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# Abstract

## Background

Evaluation of biomarkers in the context of ARDS is used to detect the presence of endothelial and/or alveolar epithelial injuries. Angiopoietin-2 (Ang-2), soluble intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion protein-1 (VCAM-1), P-selectin and E-selectin are biomarkers of endothelial injury, whereas the receptor for advanced glycation end-products (RAGE) reflects alveolar epithelial injury. The aim of this study was to evaluate whether the trends in plasma concentration of the biomarkers mentioned above were different in survivors and non-survivors of COVID-19-related ARDS. Furthermore, we compared the expression of biomarkers of vascular and endothelial injury in patients with COVID-19-related ARDS and classical ARDS.

## Methods

This prospective study was performed in two COVID-19 dedicated Intensive Care Units (ICU) and one non-COVID-19 ICU at Ferrara University Hospital. A cohort of 31 mechanically ventilated patients with COVID-19 ARDS and a cohort of 10 patients with classical ARDS were enrolled. Ang-2, ICAM-1, VCAM-1, P-selectin, E-selectin and RAGE were determined with a bead-based multiplex immunoassay at three time points: inclusion in the study (T1), after  $7\pm 2$  days (T2) and  $14\pm 2$  days (T3). The primary outcome was to evaluate the plasma trend of the biomarker levels in survivors and non survivors. The secondary outcome was to evaluate the differences in respiratory mechanics variables and gas exchanges between survivors and non survivors. Furthermore, we compared the plasma levels of the biomarkers at T1 in patients with COVID-19-related ARDS and classical ARDS.

## Results

In COVID-19-related ARDS, the plasma levels of Ang-2 and ICAM-1 at T1 were statistically higher in non-survivors than survivors, ( $p=0.04$  and  $p=0.03$ , respectively) whereas those of P-selectin, E-selectin and RAGE did not differ. Ang-2 and ICAM-1 at T1 were predictors of mortality (AUROC 0.650 and 0.717, respectively). At T1, RAGE and P-selectin levels were higher in classical ARDS, than in COVID-19-related ARDS. Ang-2, ICAM-1 and E-selectin were lower in classical ARDS than in COVID-19 related ARDS (all  $p<0.001$ ).

## Conclusions

COVID-19 ARDS is characterized by an early pulmonary endothelial injury, as detected by Ang-2 and ICAM-1. COVID-19 ARDS and classical ARDS exhibited a different expression of biomarkers, suggesting different pathological pathways.

**Trial registration:** NCT04343053

**Date of registration:** April 13, 2020

# Background

Most critically ill patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) develop a syndrome that fulfills the Berlin criteria for the acute respiratory distress syndrome (ARDS) [1]. Nonetheless, some authors advocate that significant differences exist between COVID-19-related ARDS and classical ARDS, based on studies showing higher respiratory system compliance [2, 3] and lower recruitability [4–6] compared to classical ARDS. On the other hand, several authors do not recognize differences between classical and COVID-19-related ARDS based on the results of a large observational studies suggesting similar physiological features and outcomes [7].

The pathogenesis of classical ARDS acknowledges an association between “endothelial” and “alveolar” injury, but the impact of the two entities differs between different ARDS phenotypes [8]. Recently, some morphological pathways of the COVID-19-related ARDS have been elucidated in a series of autopsies. The histologic analysis of pulmonary vessels showed widespread thrombosis with microangiopathy [9], diffuse alveolar damage, capillary congestion, necrosis of pneumocytes, interstitial and intra-alveolar oedema and platelet–fibrin thrombi [10]. In classical ARDS, specific biomarkers are useful to provide pathogenetic and prognostic insights of the syndrome. [11–14]. Biomarkers evaluation can identify the presence of endothelial and alveolar epithelial injury. Angiopoietin-2 (Ang-2), soluble intercellular adhesion molecule-1 (ICAM-1), soluble vascular cell adhesion molecule-1 (VCAM-1), P-selectin, E-selectin are used as biomarkers of endothelium injury, whereas receptor for advanced glycation end-products (RAGE) is a marker for alveolar epithelial injury [13].

Regarding COVID-19-related ARDS, besides studies focused on markers of systemic endothelial dysfunction, such as D-dimers [3], few data are available on other biomarkers, despite the urgent need of understanding if the “epithelial” and/or the “endothelial” injury are similar or different to classical ARDS.

The aim of this study was to evaluate whether the plasma levels of “endothelial” and “alveolar” biomarkers (Ang-2, ICAM-1, VCAM-1, P selectin, E-selectin and RAGE) vary over time between survivors and non-survivors in COVID-19-related ARDS patients. Furthermore, we compared the biomarkers expression in COVID-19-related and classical ARDS.

## Methods

### Study design

The present analysis is based on data from the Pro-thrombotic Status in Patients With SARS-Cov-2 Infection (ATTAC-Co) study (ClinicalTrials.gov Identifier: NCT04343053). The ATTAC-co was a prospective, single-centre study performed at the University Hospital of Ferrara (Italy). The present analysis is specifically designed to investigated the relationship between several biomarkers that are indicators of epithelial and endothelial lung injury in consecutive patients with confirmed COVID-19 who were admitted to COVID-19 dedicated Intensive Care Unit between April and June 2020 and needed mechanical ventilation. A group of patients, admitted in the same period in the non-COVID dedicated ICU,

with similar clinical characteristics in terms of ARDS presentation, but negative for SARS-CoV-2 infection were also included as controls. The protocol was approved by the Ethics Authority (Comitato Etico di Area Vasta Emilia Centro, Bologna, Italy). All patients gave their written informed consent. In case of unconsciousness, the informed consent was signed by their next of kin or legal authorized representative.

## Study population

Inclusion criteria were: a) age > 18 years; b) confirmed SARS-CoV-2 infection; c) need of invasive mechanical ventilation; d) meeting the Berlin criteria definition for ARDS. Patients were excluded from the study in case of pregnancy or do-not-resuscitate order. SARS-CoV-2 infection was confirmed by reverse-transcriptase-polymerase-chain-reaction assay (Liaison MDX, Diasorin, Saluggia, Italy) from nasopharyngeal swab specimen or tracheal aspirate. Clinical management was in accordance with current guidelines and specific recommendations for the COVID-19 pandemic by Health Authorities and Scientific Societies [15].

## Procedures and blood samples

At ICU admission, clinical and physiological variables were collected: age, sex, body mass index (BMI), Sequential Organ Failure Assessment (SOFA) Score, Simplified Acute Physiology Score (SAPS) II, comorbidities and main laboratory data. Respiratory data collected were: ratio of partial pressure of arterial oxygen to fractional concentration of inspired oxygen ( $PaO_2/FiO_2$ ), partial pressure of carbon dioxide ( $PaCO_2$ ), end-inspiratory plateau pressure (assessed performing a 5 s end-inspiratory occlusion), positive end expiratory pressure (PEEP), tidal volume for predicted body weight ( $V_t/PBW$ ) and static compliance of the respiratory system calculated as tidal volume/ (end inspiratory plateau pressure – total PEEP),

Three different samples of venous blood were collected: at the inclusion in the study (T1), after  $7 \pm 2$  days (T2), and  $14 \pm 2$  days (T3). Blood withdrawn was performed from an antecubital vein using a 21-gauge needle. All patients underwent blood sampling in the early morning. The first 2 to 4 mL of blood were discarded. The serum and plasma samples were stored at  $-80^\circ C$ . The plasma levels of Ang-2, ICAM-1, VCAM-1, P-selectin, E-selectin, and RAGE, were determined with a bead-based multiplex immunoassay (Luminex, Thermo Fisher Scientific, Waltham, MA, USA). The latter laboratory analyses were performed in the Translational Research Center of the Maria Cecilia Hospital, Cotignola (RA), Italy.

Simultaneously to each blood sample, gas exchanges and respiratory mechanics variables were collected.

## Outcomes

The primary outcome was to evaluate the trend of the biomarker's plasma levels in survivors and non-survivors COVID-19 ARDS patients. The secondary outcome was the differences in respiratory mechanics variables and gas exchanges between survivors and non-survivors. Furthermore, we compared the biomarkers' plasma levels at T1 in patients with COVID-19-related ARDS and classical ARDS. Finally, we compared clinical characteristics and plasma levels of the biomarkers at ICU admission between patients

with COVID-19 related ARDS and classical ARDS. The dataset of classical ARDS was prospectively registered during the same study period, enrolling all consecutive ARDS patients admitted to a non-COVID-19 dedicated ICU at Ferrara Hospital.

## Statistical analysis

Continuous variables with normal distribution were expressed as mean  $\pm$  SD. Continuous variables with a non-normal distribution were expressed as median and interquartile range. Normal distribution of the variables was tested with the Kolmogorov–Smirnov test. The variables normally distributed were compared by t-test; otherwise the Mann-Whitney U was used. Categorical variables were summarized in terms of numbers and percentages and compared using the two-sided Fisher’s exact test. Differences between measurements were analyzed using repeated measures ANOVA or Two-sample Kolmogorov-Smirnov analysis for data with normal or not normal distribution, respectively. When multiple comparisons were made, p-values were adjusted by the Bonferroni post hoc procedure. Receiver operator characteristic (ROC) curves were used to analyze the biomarkers’ ability of to predict 90-day mortality. ROC curve analyses are reported as AUROC, with a 95% confidence interval (95% CI). Due to the unpredictable nature of the COVID-19 outbreak, we were unable to assume an “a priori” sample size; as a convenience sample size, we enrolled all consecutive patients with confirmed COVID-19 who were admitted to two COVID-19 dedicated Intensive Care Unit between April and June 2020.

For all comparisons, a p-value of  $\leq 0.05$  was considered statistically significant. When appropriate, 95% confidence intervals (CIs) were calculated. All analyses were performed with SPSS 25 (IBM, USA).

## Results

### *Populations*

Thirty-one mechanically ventilated patients with COVID-19-related ARDS were included in the study. Patients were mostly male (26/31, 84%), and the most common comorbidities were hypertension (17/31, 42%) and chronic kidney disease (8/31, 26%). Thirteen patients (43%) were successfully weaned within 28-days and the mean length of stay in ICU was 31 [25–42] days. Hospital mortality was 35% (11 non-survivors). Non-survivors were older ( $68 \pm 6$  vs  $61 \pm 6$ ; p value = 0.05) than survivors whereas other baseline characteristics were not significantly different (Table 1).

Table 1  
Baseline characteristics and values of markers of lung injury in survivor vs non survivors.

<b>Variables</b>	<b>Survivors (n = 20)</b>	<b>Non survivors (n = 11)</b>	<b>p value</b>
Age, years	61 ± 6	68 ± 6	0.05
Male, sex, no. %	17 (85)	9 (82)	0.59
BMI, Kg/m <sup>2</sup>	27.8 ± 4.1	28.9 ± 3.2	0.41
SAPS II at ICU admission	27 [21–38]	31 [21–37]	0.55
SOFA score at ICU admission	4 [2–5]	5 [3–6]	0.23
Length of ICU stay (days)	31 [25–43]	24 [16–31]	0.04
<b>Comorbidities</b>			
Hypertension, no. (%)	9 (45)	8 (73)	0.13
Dyslipidemia, no. (%)	4 (20)	1 (9)	0.40
Former smoker, no. (%)	4 (20)	6 (54)	0.06
Diabetes, no. (%)	4 (20)	1 (9)	0.40
COPD, no. (%)	2 (5)	2 (18)	0.45
Chronic kidney disease, no. (%)	3 (15)	5 (45)	0.08
<b>Laboratory data at inclusion</b>			
White blood cells, x10 <sup>3</sup> /L	9.8 [7.5–12.9]	10.2 [8.8–12.7]	0.85
Lymphocytes, x 10 <sup>3</sup> /L	965 [640–1167]	680 [560–980]	0.18
Haemoglobin, g/dL	12.1 ± 1.8	13.1 ± 1.1	0.11
Platelets count, x10 <sup>3</sup> /L	286 [265–386]	286 [195–332]	0.43
apTT, seconds	39 ± 5	37 ± 5	0.34
INR	1.1 ± 0.1	1.1 ± 0.1	0.35
Fibrinogenm mg/dL	708 [655–888]	786 [532–862]	0.87
D-dimer, mcg/mL	31 [14–42]	36 [19–57]	0.31
IL-6, pg/mL	84 [17–149]	60 [37–145]	0.73
<b>Respiratory variables at admission</b>			

P-value: for the comparison between survivors vs non survivors cases. Data are reported as number (percentage), mean ± standard deviation or median [interquartile range] as appropriate. BMI: body mass index. COPD: chronic obstructive disease.

Variables	Survivors (n = 20)	Non survivors (n = 11)	p value
PaO <sub>2</sub> / FiO <sub>2</sub> ratio	183 [124–264]	116 [81–184]	0.045
PaCO <sub>2</sub> , mmHg	48 [36–56]	48 [41–69]	0.40
V <sub>T</sub> /PDW, mL	6.0 ± 0.5	6.0 ± 0.5	0.96
Driving Pressure, cmH <sub>2</sub> O	9 [8–11]	8 [7–14]	0.79
Compliance Respiratory System	57 [36–78]	60 [51–64]	0.95
Plateau pressure, cmH <sub>2</sub> O	18 [15–21]	21 [18–23]	0.11
PEEP setting (cmH <sub>2</sub> O)	12 [9–12]	10 [8–11]	0.07
P-value: for the comparison between survivors vs non survivors cases. Data are reported as number (percentage), mean ± standard deviation or median [interquartile range] as appropriate. BMI: body mass index. COPD: chronic obstructive disease.			

At ICU admission, the PaO<sub>2</sub>/FiO<sub>2</sub> ratio was higher in survivors (183 [126–264]) compared to non-survivors (116 [81–184]); p value = 0.04), while there were no differences in other PaCO<sub>2</sub> or respiratory mechanics variable (Table 1). D-dimer levels did not differ at ICU admission between survivors and non-survivors (31 [14–42] vs 36 [19–57], p = 0.31) but there was an increase over time in non-survivors (p = 0.006 for two-sample Kolmogorov-Smirnov; Figure S1).

### *Biomarkers*

In COVID-19-related ARDS Ang-2 was higher in non-survivors than in survivors at ICU admission (p = 0.04), and decreased similarly over time in the two groups (p = 0.17 for two-sample Kolmogorov-Smirnov analysis) (Fig. 1, Fig. 2, Table S1). The area under the receiver operating characteristic curve (AUROC) of Ang-2 at ICU admission for hospital mortality was 0.650. ICAM-1 values were higher in non-survivors than survivors (p = 0.03 at ICU admission, Fig. 1), and repeated measure analysis showed more significant decrease from T1 to T3 in survivors compared to non-survivors (p = 0.03 for two-sample Kolmogorov-Smirnov analysis) (Fig. 2, Table S1) The AUROC of ICAM-1 for hospital mortality at ICU admission was 0.717. The ICAM-1 plasma level at ICU admission was inversely correlated with the worsening of respiratory system compliance over time (r=-0.470; p = 0.03). VCAM-1 levels at T1 were higher in non-survivors than in survivors, though not statistically significantly (p = 0.06) (Fig. 1). We did not find differences in P-selectin or E-selectin plasma levels at ICU admission or during ICU stay between survivors and non-survivors.

In the overall study population, RAGE decreased significantly during the study period (60.9 [18.8–274.4] at T1, 30.6 [13.4–90.7] at T2, and 20.5 [12.2–41.6] at T3; p value T3 vs T1 < 0.001). RAGE did not differ between survivors and non-survivors at ICU admission (p = 0.34), (Fig. 1, Fig. 2) and had similar decrease overtime. (p = 0.71 for two-sample Kolmogorov-Smirnov analysis) (Table S1).

### *Gas exchange and respiratory mechanics*

Measures of gas exchange and respiratory mechanics during the study period are reported in Table 2. At ICU admission, the  $\text{PaO}_2/\text{FiO}_2$  ratio was significantly higher in survivors than in non-survivors ( $p = 0.04$ ), and the  $\text{PaO}_2/\text{FiO}_2$  ratio increased more in survivors than in non-survivors from T1 to T3 ( $p = 0.001$  for two-sample Kolmogorov-Smirnov analysis) (Table 2).  $\text{PaCO}_2$  values did not differ at ICU admission between survivors and non-survivors ( $p = 0.40$ ), but the  $\text{PaCO}_2$  increased over time more in non-survivors than in survivors ( $p = 0.001$  for two-sample Kolmogorov-Smirnov analysis from T1 to T3). Finally, the respiratory system compliance and the plateau pressure did not significantly differ among the two groups.

Table 2  
Gas exchange and respiratory mechanics in mechanically ventilated COVID-19 ICU patients.

	<b>T1</b> <b>n = 31</b>	<b>T2</b> <b>n = 26</b>	<b>T3</b> <b>n = 20</b>	<b>p- value</b> <b>for inter-group</b> <b>trend</b>	<b>p value for group trend</b> <b>comparison</b>
<b>PaO<sub>2</sub>/F<sub>i</sub>O<sub>2</sub></b>					
Survivor	183 [124–264]	215 [165–304]	251 [204–355]	0.08	0.001
Non survivor	116 [81–184]	104 [77–147]	144 [94–193]	0.84	
<b>PaCO<sub>2</sub></b>					
Survivor	48 [36–56]	45 [38–67]	41 [35–55]	0.22	0.001
Non survivor	48 [41–69]	63 [50–71]	64 [54–83]	0.02	
<b>Driving Pressure</b>					
Survivor	9 [8–11]	8 [7–11]	8 [6–8]	0.31	0.10
Non survivor	8 [7–14]	10 [9–12]	10 [9–12]	0.57	
<b>Static compliance</b>					
Survivor	57 [36–78]	71 [41–80]	60 [49–79]	0.07	0.35
Non survivor	60 [51–64]	41 [24–54]	46 [40–64]	0.23	
<b>PEEP</b>					
Survivor	12 [9–12]	8 [6–11]	8 [6–10]	0.36	0.03
Non survivor	10 [8–11]	14 [9–17]	10 [9–13]	0.93	
<b>Plateau</b>					
Survivor	18 [15–21]	17 [14–22]	15 [12–17]	0.25	0.07
Non survivor	21 [18–23]	22 [19–31]	21 [16–23]	0.84	
Data are reported as median [interquartile range]					

*Comparison between COVID-19-related ARDS and classical ARDS*

All the biomarkers analyzed differed significantly between COVID-19-related ARDS and classical ARDS at ICU admission (Table 3). In detail, Ang-2, ICAM-1 and E-selectin were higher in COVID-19 related ARDS (all  $p < 0.001$  for group comparison), whereas RAGE and P-selectin levels were higher in classical ARDS. A comparison of clinical characteristics between classical ARDS and COVID-19-related ARDS patients is shown in Table S2. Patients with classical ARDS had higher hemoglobin and lower D-Dimer and international normalized ratio (INR) when compared to COVID-19 patients (Table S2). Regarding respiratory mechanics, patients with classical ARDS had higher driving pressure, lower respiratory system compliance, and were ventilated with higher PEEP levels (Table S2).

Table 3  
Comparison of markers of endothelial and epithelial dysfunction between patients with COVID-19-related ARDS and classical ARDS

<b>Biomarker</b>	<b>Covid-19-related ARDS (n = 31)</b>	<b>Classical ARDS (n = 10)</b>	<b>p value</b>
RAGE, pg/mL	60 [18–274]	789 [440–1021]	< 0.001
ICAM-1, ng/mL	1093 [575–1515]	75.7 [63.1–89.6]	< 0.001
VCAM-1, ng/mL	1093 [575–1515]	739 [439–1021]	0.019
Ang-2, pg-mL	3909 [1658–6348]	1045 [627–1654]	< 0.001
P-selectin, ng/mL	93 [50–145]	750 [631–1103]	< 0.001
E-selectin, ng/mL	24.9 [19.2–42.5]	3.3 [2.4–5.7]	< 0.001
Data are reported as median [interquartile range].			

## Discussion

Our study shows that some biomarkers of pulmonary endothelial injury, Ang-2 and ICAM-1, are significantly higher in non-survivors patients with COVID-19 related ARDS than survivors. Conversely, the levels of an established biomarker of alveolar epithelial injury, the RAGE, were not different. Our findings suggest that the endothelial injury pathway may be predominant in the pathogenesis of more severe forms of COVID-19-related ARDS. Also, study highlights substantial differences in the biomarkers levels expressed in COVID-19-related ARDS and classical ARDS, supporting a different pathophysiological pathway between these two syndrome, as recently suggested [2, 16]

Previous studies have shown that higher levels of Ang-2 are related to an increased pulmonary vascular leak, [17, 18] and that the pulmonary vascular endothelium to up-regulates the ICAM-1 expression in response to inflammation. [4, 12]. Other authors found that ICAM-1 can bind alveolar macrophages and enhance inflammatory cytokine production in the alveoli [19]. We found that the plasma levels of Ang-2 and ICAM-1 at T1 were higher in non-survivors than in survivors and, furthermore. i.e. that the plasma levels of Ang-2 and ICAM-1 increased rapidly in COVID-19 non-survivors, within 24 hours from ICU

admission. To this end, our data might suggest that in COVID-19 ARDS patients the extent of pulmonary endothelium injury, as reflected by ICAM-1 levels, sustains the overall pulmonary inflammation and contribute to the pathogenesis of alveolar epithelial injury. Of note, we found a correlation between ICAM-1 level at admission and worsening of respiratory system compliance during the ICU stay. We thus speculate that evolution of COVID-19 related ARDS towards a more severe phenotype could be related to the extent of pulmonary endothelium injury in the early phase of the disease. Remarkably, the D-dimer levels, a widely used prognostic marker in COVID-19 patients, [3, 20, 21] did not differ between survivors and non-survivors within 24 hours from ICU admission.

We were unable to detect any differences in RAGE levels between survivors and non-survivors at ICU admission and throughout the ICU stay. Moreover, the RAGE levels were lower than those previously described in the context of classical ARDS [13]. Since higher RAGE levels have been previously associated with clinical outcomes in patients with classical ARDS, several considerations should be made: *firstly*, reduced RAGE expression has been reported in patients with idiopathic pulmonary fibrosis, and a high prevalence of lung fibrosis is suspected in COVID-19-related ARDS survivors [22]; *secondly*, it has been hypothesized that decreased circulating RAGE could be a marker of deficient inflammatory control [23]. *Finally*, low plasma RAGE were described in respiratory failure due to COPD [24]. RAGE levels may therefore reflect differing mechanisms of lung injury in different lung diseases, and in our study seems to confirm that in COVID-19 ARDS the pulmonary endothelial injury is predominant when compared to alveolar injury.

When comparing COVID-19-related ARDS with a cohort of patients with classical ARDS, we observed a significant difference in all the evaluated biomarkers. Ang-2 and ICAM-1 were higher in COVID-19-related ARDS, further highlighting the role of pulmonary vascular injury in this context. On the other hand, RAGE was higher in classical ARDS patients. P-selectin was higher in classical ARDS patients whereas E-selectin was higher in COVID-19-related ARDS. (Table 3). The different behavior of these two-selectin markers further highlights the predominant role of the endothelium, the primary source of E-selectin, [25] in the genesis of COVID-19-related ARDS.

This is a hypothesis-generating study and we must acknowledge some limitations. First, this is a single-centre prospective study focused on the description of pathological alteration in COVID-19-related ARDS. The clinical relevance of these findings should be confirmed in future interventional studies. Secondly, we enrolled patients needing mechanical ventilation and thus our results could not be extended to mild or moderate COVID-19.

## Conclusions

The most severe forms of COVID-19 ARDS are characterized by the predominance of the “endothelial” over the “alveolar” injury, as detected by the higher levels of Ang-2 and ICAM-1 in non-survivors compared to survivors; COVID-19 ARDS and classical ARDS had similar loss in gas exchange, but exhibited a different expression of biomarkers, suggesting different pathological pathways.

# Abbreviations

Ang-2: angiopoietin-2; ARDS:acute respiratory distress syndrome; COVID-19:Coronavirus disease 2019; ICAM-1:soluble intercellular adhesion molecule-1; ICU:intensive care unit; RAGE:soluble receptor for advanced glycation end-products; SARS-CoV-2:severe acute respiratory syndrome coronavirus 2; E-selectin:soluble E-selectin; VCAM-1:soluble vascular cell adhesion molecule-1

# Declarations

## Ethics approval and consent to participate

The study was approved by the local ethics committee (Comitato etico Indipendente di Area Vasta Emilia Centro), protocol number 339/2020, date of approval 07<sup>th</sup> April 2020).

## Consent for publication

Not applicable

## Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests

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The study was supported by the University of Ferrara, Ferrara, Italy.

## Authors' contributions

SS, AF, GC and MC were involved in the conception and the design of the study, analyzed the data, and wrote the paper. OZ, MV, IO, VS collected the data. TT and AF performed the statistical work. EM, FVD, FF, PR, RF, AP contributed to the analysis of the data; SG, FM, RP and CAV contributed to the critical revision of the manuscript for important intellectual content.

All authors reviewed the manuscript and agreed with the final version.

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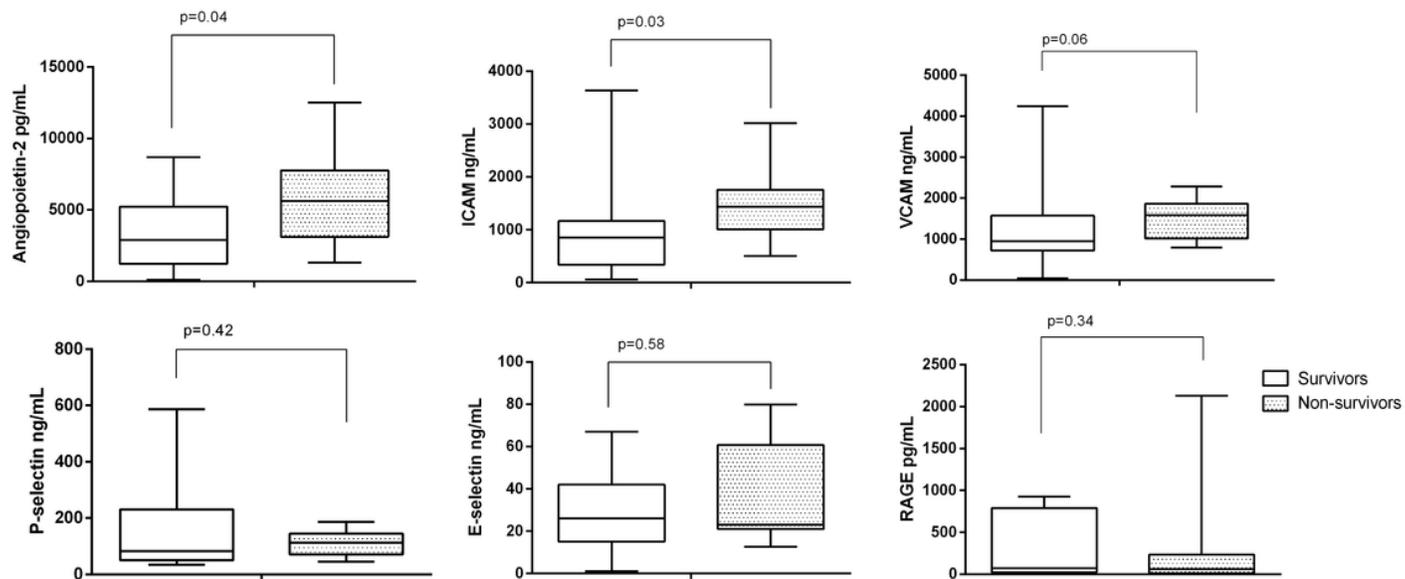
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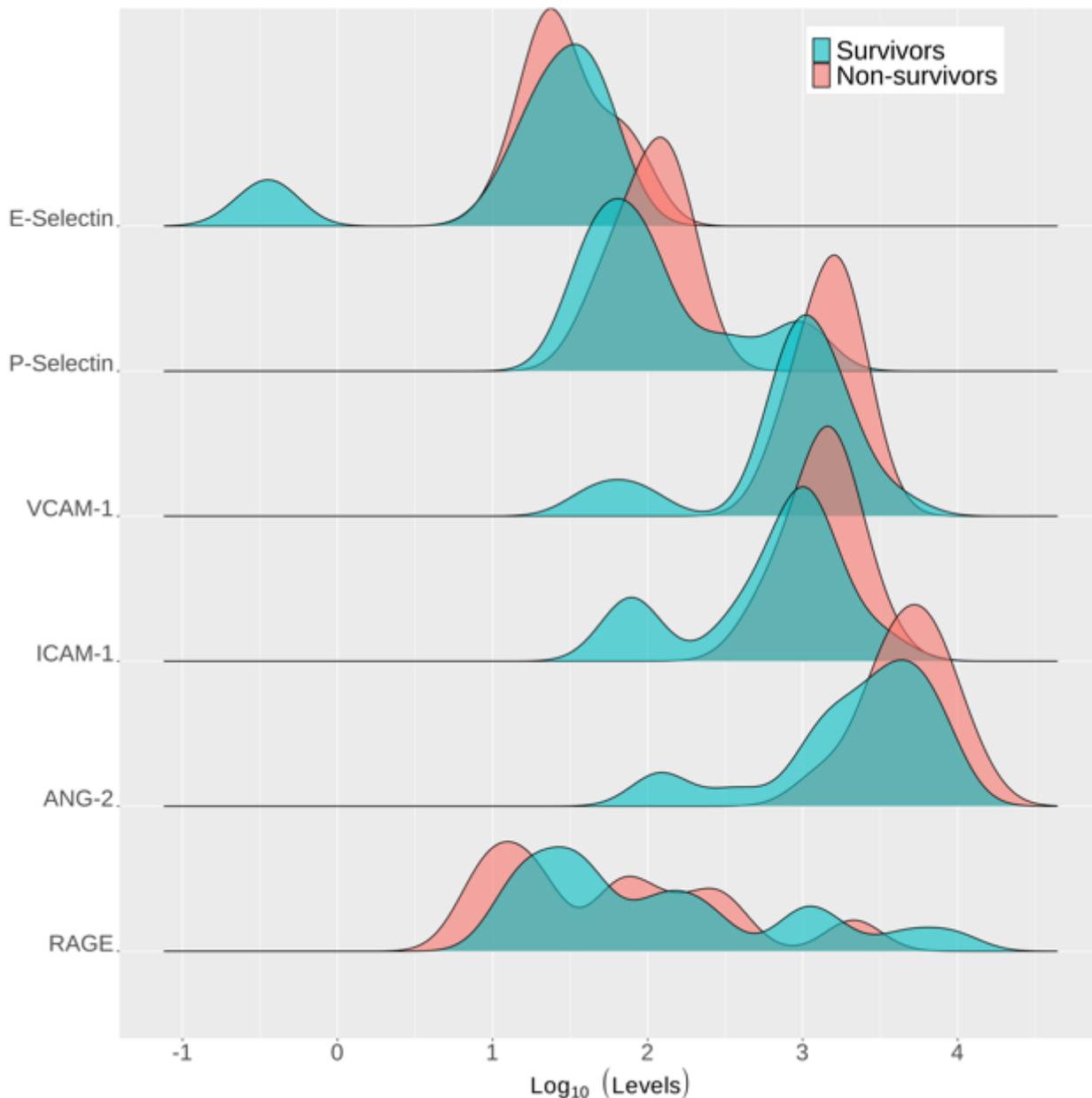
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## Figures



**Figure 1**

Box-and-whisker plot for comparison of the biomarkers in survivors (n=20) and non-survivors COVID-19 (n=11) patients at the inclusion of the study.



**Figure 2**

Density distribution of six mediators; RAGE, ANG-2, ICAM-1, VCAM-1, P-Selectin, E-Selectin measured in serum samples collected at timepoint 1. The distribution is colored according to patient outcome, where red relates to patients who died, while blue denotes patients who recovered (survivors, n=20). The horizontal axis represents the  $\text{Log}_{10}$  levels of mediators measured in pg/mL for RAGE and ANG-2, and in ng/mL for ICAM-1, VCAM-1, P-Selectin and E-Selectin. The vertical axis corresponds to the respective mediators. Figure generated using R statistical software, version 4.0.2.

## Supplementary Files

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