Distinct temporal characteristics of circulating alveolar epithelial and endothelial injury markers in ARDS with COVID-19: a preliminary retrospective report

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Background

In the most severe cases, Coronavirus disease (COVID-19) leads to acute respiratory distress syndrome (ARDS) that is characterized by alveolar epithelial and endothelial injuries [1,2]. Evaluating the temporal changes of established alveolar tissue injury markers[3] can provide insights regarding the time course and specific contribution of alveolar epithelial and of endothelial injury to the pathogenesis of COVID-19 ARDS. Recently, Spadaro et al. have reported that COVID-19 ARDS is characterized by the increases of circulating endothelial injury markers[4]. However, the detailed temporal characteristics of changes in these markers are still not clear. In this preliminary study, we investigated the levels of a circulating alveolar epithelial injury marker; soluble receptor for advanced glycation end-products (sRAGE), an endothelial injury marker; angiopoietin-2 (ANG-2), and an alveolar barrier permeability indicator; surfactant protein D (SP-D) in serum from COVID-19 patients with or without ARDS.

Methods

The patients diagnosed as COVID-19 by real-time polymerase chain reaction and admitted to Yokohama City University Hospital from January to August 2020 were included in this retrospective observational study (ethics reference number: [417x767]. CC-BY-NC 4.0 International licenseIt is made available under a (which was not certified by peer review) doi: medRxiv preprint
Concentrations of sRAGE, ANG-2, and SP-D in the residual serum samples were measured using enzyme-linked immunosorbent assay kits (human RAGE: DY1145, human ANG-2: DY623, human SP-D: DY1920, R&D systems, MN, USA). We compared the concentrations of these markers on the first or second hospital day between ARDS and non-ARDS patients. Moreover, we analyzed temporal changes of these markers during the first eight hospital days in ARDS patients.

The data between ARDS and non-ARDS patients were compared with the Mann-Whitney U test. Temporal changes of the markers were analyzed with Friedman test and post-hoc Dunn’s test. The peak day of the each of the markers were analyzed with Kruskal-Wallis test and post-hoc Dunn’s test. All statistical analyses were performed using Prism 9.0 software (Graphpad Software, CA, USA). Statistical significance level was set at P < 0.05.

**Results**

Eleven ARDS and ten non-ARDS patients, all with COVID-19, were included in the study. Patient characteristics are shown in Table 1. The diagnosis of ARDS was made on the first or second hospital day. The initial serum levels of sRAGE and SP-D were
significantly higher in the ARDS group than in the non-ARDS group, however the
ANG-2 level was not (Table.1).

Analysis of the temporal changes of these markers in ten ARDS patients excluding one
patient with missing data revealed that serum sRAGE level peaked just after admission,
and it gradually decreased with hospital days (Fig.1). In contrast, serum ANG-2 and
SP-D did not significantly decrease during first eight hospital days and the peak timings
of these markers were during a later disease stage (Fig.1).

Discussion

The present study indicates that alveolar epithelial injury occurring at the very early
disease stage, which is indicated by the increase in sRAGE level, is a hallmark of
COVID-19 ARDS pathogenesis. On the other hand, levels of ANG-2 peaked at later
time points, suggesting that the endothelial injury continue to exacerbate for several
days after admission. Moreover, the trajectory of ANG-2 level, rather than of sRAGE
level, was parallel with the trajectory of SP-D, an indicator of alveolar barrier
permeability. These data suggest that the endothelial injury contribute to the
exacerbation of the alveolar barrier disruption during the later disease stage.
The difference in the peak timing of epithelial and endothelial injury markers suggests the distinct mechanisms for injury to each type of cell. A hypothesis generated from our results is that the initial alveolar epithelial injury might be a trigger of the subsequent endothelial injury. To obtain insights into the pathogenesis of alveolar tissue injury during COVID-19, further studies are warranted.

List of abbreviations

ARDS: acute respiratory distress syndrome; COVID-19: coronavirus disease; sRAGE: soluble receptor for advanced glycation end-products; ANG-2: angiopoietin-2; SP-D: surfactant protein D; SARS-CoV-2: severe acute respiratory syndrome coronavirus-2
Declarations:

Ethics approval and consent to participate:

The study protocol was reviewed and approved by the institutional review board of Yokohama City University Hospital (approval number: B200700100). The need for informed consent was waived by the institutional review boards because of the retrospective observational design of the study.

Consent for publication:

Not applicable.

Availability of data and materials

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

Competing interests

The authors have disclosed that they do not have any potential competing interest.

Funding:
The authors have declared no specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Authors' contributions:

KT conducted the study, performed ELISA, analysed data, and wrote the manuscript.

NY performed ELISA and reviewed the manuscript. TM supervised statistical data analysis and reviewed the manuscript. MA collected patients’ clinical data and reviewed the manuscript. TG supervised the study and reviewed the manuscript.

Acknowledgements

The authors wish to thank Department of Emergency Medicine (Prof. Ichiro Takeuchi), Department of Microbiology (Prof. Akihide Ryo), and Yokohama City University Center for Novel and Exploratory Clinical Trials for collecting and providing the blood samples.
References

1. Polak SB, Gool ICV, Cohen D, Thüsen JH von der, Paassen J van. A systematic review of pathological findings in COVID-19: a pathophysiological timeline and possible mechanisms of disease progression. Modern Pathol. 2020;33:2128–38.

2. Deshmukh V, Motwani R, Kumar A, Kumari C, Raza K. Histopathological observations in COVID-19: a systematic review. J Clin Pathol. 2020;jclinpath-2020-206995.

3. Zee P van der, Rietdijk W, Somhorst P, Endeman H, Gommers D. A systematic review of biomarkers multivariately associated with acute respiratory distress syndrome development and mortality. Crit Care. 2020;24:243.

4. Spadaro S, Fogagnolo A, Campo G, Zucchetti O, Verri M, Ottaviani I, et al. Markers of endothelial and epithelial pulmonary injury in mechanically ventilated COVID-19 ICU patients. Crit Care. 2021;25:74.
### Table 1

|                              | non-ARDS (n=10) | ARDS (n=11) | p-Value |
|------------------------------|-----------------|-------------|---------|
| **Age, median (IQR), years** | 57 (30-72)      | 69 (64-76)  | 0.2428  |
| **Males/Females**            | 8/2             | 10/1        | 0.5865  |
| **APACHE2 score**            | 7 (4.75-9.75)   | 10 (9.00-14.00) | *0.0204 |
| **P/F ratio at admission, median (IQR)** | 395 (304-454) | 155 (108-203) | *<0.0001 |
| **Mechanical ventilation use** | 0               | 11          | *<0.0001 |

**Laboratory data on admission**

|                         |                |              |         |
|-------------------------|----------------|--------------|---------|
| **WBC count**           | 4600 (2525-7925) | (5800-9600)  | 0.1567  |
| **Neutrophil Count**    | 2258 (1268-5416) | (4292-7350)  | *0.0430 |
| **Lymphocyte count**    | 1003 (674-1475)  | 643 (342-788) | *0.0048 |
| **Platelet count**      | 186 (86-317)    | 220 (176-287) | 0.4262  |
| **D-dimer**             | 0.67 (0.00-1.04) | 1.35 (0.65-2.52) | *0.0404 |
| **CRP**                 | 0.98 (0.28-2.06) | 14.62        | *<0.0001 |

* indicates statistical significance.
|                      |     |     |                   |
|----------------------|-----|-----|-------------------|
| Creatinine           | 0.75(0.63-0.87) | 0.75(0.59-0.89) | >0.9999           |
| Total bilirubin      | 0.55(0.40-0.78) | 0.60(0.50-1.00) | 0.5661            |
| Alveolar tissue injury marker levels on admission | | | |
| sRAGE                | 896(402-1718)   | 2328            | *0.0079           |
| ANG-2                | 334(65-781)     | 699(405-2201)   | 0.1321            |
| SP-D                 | 2407(772-3833)  | 16596           | *0.0062           |
Figure Legends

Figure 1

Temporal changes of (A) sRAGE, (B) ANG-2, and (C) SP-D levels in ARDS patients with COVID-19 for 8 days starting from admission. In cases where values for every 2-days were available, then an average of the values was used; where only a single day value was available, then that was used. (D) The peak day of each of the alveolar tissue injury markers. Data were presented as median ± IQR.
