Characteristics of changes in circulating markers of alveolar epithelial and endothelial injury in acute respiratory distress syndrome with COVID-19

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Abstract

The time course and specific contributions of alveolar epithelial and endothelial injury to the pathogenesis of acute respiratory distress syndrome (ARDS) with coronavirus disease (COVID-19) remain unclear. Here, we evaluated the characteristics of circulating markers of alveolar epithelial and endothelial injury in 107 serum samples from nine ARDS patients and eight non-ARDS patients, all with COVID-19. Although both the alveolar epithelial and endothelial injury markers were markedly elevated in the COVID-19 ARDS patients, our data indicate that the endothelial injury, which continues for a longer period than the epithelial injury, seems to be the main contributor to alveolar barrier disruption.

Introduction

In the most severe cases, Coronavirus disease (COVID-19) leads to acute respiratory distress syndrome (ARDS)[1] that is characterised by severe pulmonary oedema with alveolar epithelial and endothelial injuries. Several previous reports have demonstrated that the lungs from ARDS patients with COVID-19 show diffuse alveolar damage[2,3]. However, the time course and specific contribution of alveolar epithelial and of endothelial injury to the pathogenesis of COVID-19 ARDS remain unclear. Better
clarity regarding the pathogenesis will help in improved understanding of the disease, and thus, improved treatment possibilities.

There are several established circulating alveolar tissue injury markers[4]. Evaluating the temporal changes of alveolar tissue injury markers can provide insights regarding the alveolar endothelial and epithelial injuries during COVID-19 ARDS progression. Here, 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 July 2020 were included in this retrospective observational study (ethics reference number: B200700100). 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 analysed the temporal changes of these markers in ARDS patients.

Comparisons of the data between ARDS and non-ARDS patients were performed using the Mann-Whitney U test. To analyse the correlation of serum levels of sRAGE or ANG-2 with SP-D levels or PaO₂/fraction of inspired oxygen (P/F) ratios, the values of the serum markers were log-transformed to obey normal distribution, and the correlations were evaluated by linear mixed model analysis with random intercepts for individual patients. All statistical analyses were performed using R software (version 4.0.3). Statistical significance level was set at P < 0.05.

**Results**

Clinical characteristics of patients

Nine ARDS and eight non-ARDS patients, all with COVID-19, were included in the study. Patient characteristics are shown in Table 1. Three of the nine in the ARDS group died and all in the non-ARDS group survived. Among patients with ARDS, only one patient developed acute kidney injury, and some patients showed only a small increase in total-bilirubin concentration. Thus, organ dysfunction in the patients was primarily limited to the lungs.
Circulating alveolar tissue injury markers

The initial serum levels of all markers; sRAGE, ANG-2, and SP-D, were significantly higher in the ARDS group than in the non-ARDS group (Table. 1). The temporal changes in these markers in COVID-19 ARDS patients are shown in Fig.1A–C. The peak for serum sRAGE level was observed just after admission (median: day 1, IQR: day 1–3.5) (Fig.1D). In contrast, the peak timings of serum ANG-2 (median: day 4, IQR: day 2.5–6) and SP-D (median: day 5, IQR: day 3–7.5) levels were during a later phase of the disease (Fig.1D).

Linear mixed effect model analysis revealed that the serum ANG-2 levels had a significant positive correlation with the SP-D levels (fixed effect: 0.490, 95%CI: 0.184–0.797, p=0.002) (Fig. 2A) and a significant negative correlation with P/F ratios (fixed effect: -29.9, 95%CI: -56.6–-3.3, p=0.030) (Fig. 2B); however, the sRAGE levels had an opposite correlation with SP-D levels(fixed effect: -0.557, 95%CI: -0.925–-0.189, p=0.004) (Fig. 2C), and there was no significant correlation between sRAGE levels and P/F ratio (fixed effect: -19.9, 95%CI: -51.2–11.3, p=0.215) (Fig. 2D).

Discussion
Our investigation of circulating alveolar tissue injury markers indicates that alveolar barrier tissue injury is a hallmark of COVID-19 ARDS pathogenesis. Interestingly, time courses of changes in alveolar epithelial and in endothelial injury markers were different from each other. The observed serum sRAGE level was highest on admission in most patients with ARDS. In contrast, ANG-2 level reached a peak at later time points. These data suggest that the alveolar epithelial injury in the COVID-19 ARDS already reached the maximum level before hospital admission. Meanwhile, the endothelial injury seems to continue to deteriorate for several days after admission. Moreover, the ANG-2 levels were significantly correlated with the SP-D levels and P/F ratios, suggesting endothelial injury, rather than alveolar epithelial injury, might be a main contributor that leads to deterioration of alveolar permeability during ARDS with COVID-19.

Two reports have previously demonstrated that sRAGE levels in the blood of ARDS patients with bacterial sepsis also peak on the first hospital day[5,6]. Thus, alveolar epithelial injury seems to occur during the initial phase of ARDS progression irrespective of the aetiology. On the other hand, Kumpers et al. reported that circulating ANG-2 levels in patients with bacterial sepsis increased from the baseline level only in the non-survivors, not in the survivors[7]. In our study, the ANG-2 levels increased from baseline in most patients, including survivors, suggesting that SARS-CoV-2
induces more severe endothelial injury than other aetiologies, such as bacterial infection. Several reports have demonstrated that lung vascular thrombosis is a pathological feature of SARS-CoV-2[2,3]. Endothelial injury caused by SARS-CoV-2 infection might be the basal mechanism of thrombosis formation[8].

The underlying mechanism of alveolar epithelial and endothelial injury has not been fully elucidated. SARS-CoV-2 can infect alveolar type 1 and 2 epithelial cells and endothelial cells[9]. On the other hand, inflammatory responses caused by SARS-CoV-2, rather than the SARS-CoV-2 infection itself to the targeted cells, may be the main driver of the alveolar tissue injury[10]. The difference in the peak timing of epithelial and endothelial injury markers suggests that there are distinct mechanisms for injury to each type of cell.

Our data indicate that the targeting the endothelial injury rather than the epithelial injury, may be a potential efficacious approach to overcome ARDS with COVID-19. To obtain insights into the pathogenesis of alveolar tissue injury during COVID-19, further studies to confirm our results using samples from large cohorts are warranted.

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Footnotes

Contributors: 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.

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### Table.1

The clinical characteristics and serum alveolar tissue injury marker levels in ARDS and non-ARDS patients with COVID-19.

|                               | non-ARDS        | ARDS            | p-Value |
|-------------------------------|-----------------|-----------------|---------|
| Age (years)                   | 49 (30-73)      | 67 (63-75)      | 0.2634  |
| Males/Females (number)        | 6/2             | 9/0             | 0.2059  |
| APACHE2 score                 | 7 (4.25-8.75)   | 11 (9.50-15.50) | *0.0290 |
| P/F ratio at admission,       | 389 (310-412)   | 270 (152-336)   | *0.0073 |
| Mechanical ventilation use (number) | 8           | 0              | <0.0001 |
| Laboratory data on admission  |                 |                 |         |
| WBC count (/μL)               | 4900 (2700-9500) | 6200 (5400-7350) | 0.7035  |
| Lymphocyte count (/μL)        | 1109 (965-1569) | 643 (321-760)   | *0.0003 |
| Platelet count (×10^3/μL)     | 190 (120-306)   | 206 (170-256)   | >0.9999 |
| D-dimer (μg/mL)               | 0.33 (0.05-0.85) | 1.22 (0.65-7.135) | 0.0545  |
| CRP (mg/dL)                   | 0.82 (0.15-1.26) | 14.62 (8.80-16.82) | *0.0002 |
| Creatinine (mg/dL)            | 0.72 (0.60-0.84) | 0.79 (0.67-0.91) | 0.3212  |
| Total bilirubin (mg/dL)       | 0.55 (0.43-0.70) | 0.90 (0.50-1.00) | 0.3788  |
| Alveolar tissue injury marker levels on admission | | | |
| sRAGE (pg/mL)                | 701 (344-1148.0) | 2680 (1522-5076) | *0.0152 |
|                | Median (IQR)         |       |       |
|----------------|----------------------|-------|-------|
| ANG-2 (pg/mL)  | 231 (64-584)         | 699 (410-2501) | *0.0464 |
| SP-D (pg/mL)   | 1771 (458-204)       | 17542 (7423-22979) | *0.0274 |

**Figure Legends**

**Figure.1**

Temporal changes of (A) sRAGE, (B) ANG-2, and (C) SP-D levels in ARDS patients with COVID-19 for 14 days starting from admission. (D) The peak day of each of the alveolar tissue injury markers. Data were presented as median ± IQR.

**Figure.2**

The correlations between (A) ANG-2 and SP-D levels, (B) ANG-2 levels and P/F ratios, (C) sRAGE and SP-D levels, and (D) sRAGE levels and P/F ratios. Data were analyzed by linear mixed model analysis with random intercepts for individual patients. Each different colour represents the data from the individual patient.
The peak day

Day

serum sRAGE level (pg/mL)

median ± IQR

Individual data

1 × 10^5
1 × 10^4
1 × 10^3
1 × 10^2
1 × 10^1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Day

serum ANG2 level (pg/mL)

1 × 10^5
1 × 10^4
1 × 10^3
1 × 10^2
1 × 10^1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Day

serum SP-D level (pg/mL)

1 × 10^7
1 × 10^6
1 × 10^5
1 × 10^4
1 × 10^3
1 × 10^2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Day

The peak day

sRAGE  ANG-2  SP-D
A. LogSPD vs. LogANG2

B. PFratio vs. LogANG2

C. LogSPD vs. LogRAGE

D. PFratio vs. LogRAGE

P-values:
- A: P=0.002
- B: P=0.030
- C: P=0.004
- D: P=0.215