Serum surfactant protein A and D may be novel biomarkers of COVID-19 pneumonia severity

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Abstract

Background: COVID-19 is currently undergoing a pandemic worldwide, including in Japan, and many lives have been lost. So, there is an urgent need to develop new biomarkers for estimating progression or prognosis of COVID-19 patient. Lung-specific serum biomarkers, SP-A, SP-D and KL-6 have been used clinically for diagnosis of interstitial lung disease (ILDs), but their use in COVID-19 has not been investigated. To determine whether serum levels of SP-A, SP-D and KL-6 correlate with the severity of COVID-19 as indicated by clinical symptoms and radiological findings.

Methods: In a cohort of 46 consecutive COVID-19 patients, laboratory data including serum SP-A, SP-D and KL-6 concentrations of 82 blood samples were analyzed and compared between severe and non-severe cases. In addition, the disease severity as indicated by these markers and chest HRCT images were compared.

Results: Serum SP-A was significantly elevated from the early stage of pneumonia. In addition, serum SP-A and SP-D were significantly higher in severe than in non-severe cases. KL-6 was also significantly higher in severe-cases, but its mean was below the cut-off level for ILDs. AUC and their cut-off levels to detect severe cases in patients with COVID-19 infection were 0.796 for 94.9 ng/ml of SP-A, 0.827 for 116 ng/ml of SP-D and 0.640 for 275 U/ml of KL-6. HRCT image severity scores showed moderate correlations with SP-A ($\rho=0.5996$, $p<0.001$), SP-D ($\rho=0.6268$, $p<0.001$), and weak with KL-6 ($\rho=0.3489$, $p<0.001$). In the typical course of patients whose pneumonia worsened from non-severe to severe, the serum SP-A and SP-D levels increased nearly in parallel with clinical findings and HRCT images.

Conclusions: Lung-specific serum SP-A and SP-D level increased with symptom and radiological findings. These elevations can be detected from relatively early pneumonia. These results indicated that these might become a novel biomarker in COVID-19 pneumonia.

Introduction

Patients with 2019 coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), primarily present with fever, dry cough without sputum, and anosmia/hyposmia[1]. Most patients have good outcomes, but patients who are older or have compromised immunity or chronic underlying conditions may experience more serious illness[2]. Within a week of onset, some severely ill patients develop dyspnea and hypoxemia that progresses rapidly to acute respiratory distress syndrome (ARDS)[3]. No specific biomarkers are known to reflect the development of ARDS or patient prognosis.

The hydrophilic surfactant proteins A and D (SP-A and SP-D) are members of the collectin subgroup of the C-type lectin superfamily. Produced by epithelial alveolar type II cells and Club cells, these pulmonary collectins play important roles in the innate immunity of the lung[4-7] and have been used as prognostic biomarkers for interstitial lung diseases (ILDs) [8-13] and ARDS[14-16].

Because ARDS is the severe consequence of COVID-19 pneumonia, serum SP-A and SP-D concentrations may serve as biomarkers for predicting prognosis. In addition, alveolar type II cells and Club cells express the ACE2 receptor[17], which is the target receptor used by SARS-CoV-2[18] and SARS-related coronaviruses for entry into cells[19]. Based on these findings, we hypothesized that serum levels of pulmonary collectin proteins might change in relation to the severity of COVID-19 pneumonia. This retrospective, single-center study aims to determine whether SP-A and SP-D concentrations are associated with the progression and severity of COVID-19 pneumonia and whether they could be used clinically as biomarkers to guide COVID-19 treatment.

Methods

Patient selection

The enrolled 46 subjects were all COVID-19 patients who had been admitted to Sapporo Medical University Hospital between March 4, 2020 and April 20, 2020. A confirmed COVID-19 case was defined as positive for real-time reverse-transcriptase polymerase-chain reaction (RT-PCR) assay of nasal swab specimens according to the WHO guidelines. The control group comprised 22 healthy volunteers who showed no evidence of respiratory disease following a Shihoro-cho health check. The study was approved by the hospital's ethics board (approval number, 322-17). Written informed consent was waived by the Ethics Commission of the designated hospital for emerging infectious disease.

Data collection

Data including demographics, medical history, symptoms, signs, laboratory findings, and radiological images were collected from patients' medical records. Routine blood examinations included complete blood count, coagulation profile, and serum biochemical tests including renal function, liver function, creatine kinase, lactate dehydrogenase, and electrolytes. CT scans were repeatedly conducted for all inpatients. The frequency of examinations was determined by the treating physician. Although multiple blood samplings were performed on the same patient, blood test results on the day the CT was performed were extracted 1 to 4 times per person.

COVID-19 clinical classification

COVID-19 patients were separated into two groups according to severity as described in a previous report[20]. In this study, mild type and moderate type were classified as non-severe cases, and severe type and critical type were classified as severe cases.

Measurement of serum SP-A and SP-D concentration

We measured SP-A and SP-D according to the method of Shimizu et al.[21] and Nagae et al.[22], by using commercially available ELISA kits purchased from BioVendor (Brno, Czech Republic) and Yamasa Co. (Choshi, Japan), respectively. We detected KL-6 by commercially available electrochemiluminescence immunoassay for KL-6 (Sekisui Medical CO, LTD, Tokyo, Japan).

High-resolution computed tomography

High-resolution computed tomography (HRCT) images were obtained from the lung apex to the base at 5-mm intervals during suspended full inspiration with the participant in the supine position. All HRCT data were reconstructed using a high spatial-frequency algorithm and displayed on the lung parenchymal window (level, -700 Hounsfield units (HU); width, 1500 HU). HRCT images were scored for severity using a semi-quantitative scoring system designed to quantitatively estimate the pulmonary involvement of all the abnormalities based on the area involved, as described in previous studies[23, 24]. Each of the 5 lung lobes was visually scored from 0 to 5 based on % involvement as follows: 0, no involvement; 1, <5%; 2, 25%; 3, 26%–49%; 4, 50%–75%; 5, >75%. The total CT score was the sum of the individual lobar scores, with a range of 0 (no involvement) to 25 (maximum involvement). Chest CTs were evaluated by 2 pulmonologists.

Statistical analysis

All statistical analyses were performed using JMP 13.0 (SAS Institute, Cary, NC, USA) and GraphPad Prism v8 software (GraphPad, Inc., San Diego, CA, USA). The differences between groups were analyzed by non-repeated ANOVA with the Student–Newman–Keuls test or the Mann–Whitney U-test. Categorical variables were analyzed using the chi-squared test. Serum biomarkers were further analyzed via ROC curve to determine the appropriate cut-off level using JMP 13.0, allowing for optimal diagnostic accuracy. To identify correlations between two sets of data, Spearman rank correlations (r) were calculated using JMP 13.0. For all analyses, $p < 0.05$ was considered statistically significant.

Results

Patient selection and laboratory data

The study cohort included 46 consecutive COVID-19 patients admitted to Sapporo Medical University Hospital through April 20, 2020. Blood samples collected from all 46 patients (total blood samples, 82) were analyzed. Of the 46 patients, 7 were treated with ventilation plus ECMO, and 2 were treated with ventilation. Case severity was classified as severe for patients treated with oxygen therapy and non-severe for those without. The median age was 54.5 years (22–84 years), and 21 participants were male (45.6%). Most blood count and biochemical parameters, including total protein (TP), alanine aminotransferase (AST), aspartate aminotransferase (ALT), lactate dehydrogenase (LDH), and C-reactive protein (CRP), differed significantly between severe and non-severe cases (Table 1). The severe group had more patients with hypertension than did the non-severe group (33% vs 9%, respectively; $p < 0.05$). Radiologically, nearly all patients had multi-lobular ground glass opacification (GGO) with a peripheral or posterior distribution. In severe cases, ARDS-like findings, mainly consolidation, were present in images. To identify any relationships between biochemical parameters and chest CT images, the images were taken on the same day that the blood samples were drawn. The CT scores were significantly higher among severe cases than non-severe cases (16.4 ± 6.28 vs 3.6 ± 3.6 , respectively; $p < 0.001$).

Elevations in serum surfactant proteins A and D were seen early in the course of pneumonia

We observed that the serum SP-A and SP-D levels were significantly higher in severe cases than in non-severe cases (Figure 1). Additionally, SP-A was higher in non-severe cases than in healthy subjects (Figure 1).

Serum SP-A, SP-D, KL-6, LDH, CRP, and IL-6 levels were compared between non-severe cases and severe cases using ROC curves (Figure 2). Results of area under the curve (AUC) comparison of severe to non-severe cases were as follows: SP-A, 0.796; SP-D, 0.827; KL-6, 0.640; LDH, 0.970; CRP, 0.920; and IL-6, 0.879. Using ROC curves, the diagnostic cut-off levels were set at 94.9 ng/mL for SP-A, 116 ng/mL for SP-D, and 275 U/mL for KL-6. Of these cut-off levels, SP-A and SP-D were higher than the cut-off level for detecting ILDs [SP-A: 94.9 vs 45.0 ng/mL, SP-D: 116 vs 110 ng/mL, KL-6: 275 vs 500 U/mL][11, 25].

Serum SP-A and SP-D levels correlate with severity indicated by chest CT images

We examined the relationship between chest CT images and blood test parameters using the Spearman rank correlation coefficient, a nonparametric measurement to identify correlations between two sets of data. We observed that the serum levels of SP-A and SP-D correlated significantly with the CT score for each patient (Figure 3; Table 2). We observed that the serum levels of SP-A and SP-D correlated more closely with CT score than did that of KL-6.

Serum SP-A, SP-D, KL-6, LDH, CRP, and IL-6 concentrations were evaluated with respect to the disease severity indicated by chest HRCT images using ROC curves (Figure 4). The median CT score in all cases was 6, so we considered the group with a score above the median as positive. The AUCs for high vs. low CT scores were as follows: SP-A, 0.863; SP-D, 0.831; KL-6, 0.686; LDH, 0.888; CRP, 0.949; and IL-6, 0.896. The diagnostic cut-off levels using ROC curves were set at 66.1 ng/mL for SP-A, 48.5 ng/mL for SP-D, and 247 U/mL for KL-6. Of these cut-off levels, only SP-A was higher than the cut-off level for detecting ILDs [SP-A: 66.1 vs 45.0 ng/mL, SP-D: 48.5 vs 110 ng/mL, KL-6: 247 vs 500 U/mL][11, 25].

A typical case of COVID-19 progression demonstrating a correlation between serum SP-A and SP-D concentrations and disease severity

Figure 5 shows a typical severe case of COVID-19 for which serum levels of SP-A, SP-D and KL-6 were monitored regularly. After admission to our hospital, this patient exhibited worsening of symptoms and chest radiological findings (Figure 5C). Concomitant with this deteriorating condition, the serum levels of SP-A, SP-D, and other biomarkers increased (Figure 5A). The rate of increase was highest for SP-D (Figure 5B). During this period, serum KL-6 levels were slightly elevated below the cut-off level used for ILDs (500 U/mL).

Discussion

In our cohort of 46 consecutive COVID-19 patients, we observed that serum SP-A and SP-D concentrations were significantly higher in severe than in non-severe cases. The elevation in SP-A began

early in the course of pneumonia, significantly earlier than that of the lung-specific biomarker KL-6. Serum levels of SP-A and SP-D correlated strongly and positively with chest CT scores. In the typical patient whose pneumonia worsened from non-severe to severe, the serum SP-A and SP-D levels increased nearly in parallel with clinical findings and CT images. These findings suggest that serum levels of SP-A and SP-D may be useful as biomarkers to assess COVID-19 pneumonia progression.

In a previous study of potential COVID-19 biomarkers, Du R-H. et. al. indicated the elevation of troponin I or the decrease of CD3+CD8+ T cells might become the risk factors of poor prognosis[26]. Zhou F. et al. reported that elevated serum levels of IL-6, high-sensitivity cardiac troponin I, and lactate dehydrogenase, as well as lymphopenia, were more commonly seen in the most severe COVID-19 cases[27]. Biomarkers that predict the severity of COVID-19 infection in non-severe cases, representing 80% of all cases, have not been established. In addition, some authors have observed no major abnormalities in blood data in mild cases[20]. On the other hand, the elevation of SP-A and SP-D was observed relatively early in the course of pneumonia, particularly in SP-A. Consistent with our results, serum pulmonary collectin levels were reported to increase in the early stage of pneumonia and continued to increase with deterioration of the patient's condition[5]. LDH, CRP and IL-6 also increase with increasing COVID-19 severity, but the serum levels of these proteins also increase in conditions other than pneumonia and thus do not specifically reflect the pathology of COVID-19 pneumonia. Also, pulmonary collectin has been reported to increase in production under infection and to play a protective role against pathogenic viruses such as influenza A virus and herpes simplex viruses[28-30]. Thus, it is highly likely that it is also deeply involved in the infectious disease pathology of SARS-CoV-2. For the reasons above, it is presumed that it is useful as a clinical marker that reflects the pathological condition in the lung.

We would like to emphasize that serum SP-A levels in non-severe cases were significantly higher than in the control subjects (Figure 1) and many cases exceeded the cut-off levels of 45.0 ng/mL for diagnosing the presence of idiopathic pulmonary fibrosis[11]. Pulmonary collectins are normally present almost exclusively in the lungs and only leak into the blood in response to increased vascular permeability of the lungs and destruction of lung structure, conditions that are accurately reflected in COVID-pneumonia images[10]. Non-severe cases included patients with normal chest X-rays and mild pneumonia that was only visible on chest CT[20]. Thus, clinicians may be able to detect the presence of mild pneumonia when serum levels of SP-A exceed cut-off levels. In many developed countries, Japan included, CT examinations are common and can be repeated, making evaluation of the severity and progress of the disease straightforward. However, CT examination may not be available in developing countries and small clinics or when a facility has a very large number of patients. Serum pulmonary collectins can be measured in a general laboratory and can be assessed repeatedly. The observation that SP-A and SP-D levels mirror CT image findings suggests that pulmonary alveolar injury could be monitored in the absence of radiological images using these collectin proteins as biomarkers. Such measurements may assist in treatment decisions, such as whether a patient should be hospitalized or stay at home. Although this study does not address changes in these proteins during the recovery period, decreasing concentrations of serum pulmonary collectins might be associated with better clinical outcomes, as demonstrated for ILDs[13, 31].

Our study has several limitations. First, because of the retrospective observational nature of the study and the small number of cases in the cohort, the results must be confirmed in a larger study. Second, because our hospital mainly accepts the critical and mild cases, there was a bias in the patient group. These results will be further addressed in a future multi-institutional study.

In conclusion, lung-specific serum SP-A and SP-D levels increased with the aggravation of symptoms and disease severity indicated by radiological findings. Particularly SP-A levels elevated from the relatively early stage of the pneumonia. These protein levels in sera can be easily and repeatedly tested at low cost compared to that of chest CT. We propose that serum SP-A and SP-D level might be useful as biomarkers of COVID-19 pneumonia severity.

Declarations

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Authors' contributions

A. S. and K. K. developed the concept and collected the clinical data. A. S. and K. M. performed the experiments. A.S. analyzed and interpreted the data, and wrote the manuscript. K. K., S. T., H. T. and H. C. assisted with data analysis and interpretation and supervised the statistical analysis.

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Availability of data and materials: Please contact author for data requests.

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Tables

Table 1. Participant characteristics. Data are presented as the mean $\pm$ SD. The two groups were compared using the Mann-Whitney U-test. Differences between the groups regarding sex, hypertension, and diabetes mellitus were assessed using the chi-squared test.

	Non-severe cases	Severe cases	p
Number of patients (number of samples)	34 (51)	12 (31)	
Median age (years)	49.6 ± 15.7	65.1 ± 10.7	<0.001
	[51.5 (22-73)]	[61.5 (53-84)]	<0.001
Sex (male/female)	14/20	7/5	0.305
BMI	23.0 ± 4.1	24.5 ± 4.2	0.234
WBC (/L)	4994 ± 1658	3622 ± 3622	<0.001
Neutrophils (/μL)	3153 ± 1372	6436 ± 3455	<0.001
Lymphocytes (/μL)	1344 ± 579	802 ± 546	<0.001
Monocytes (/μL)	356 ± 143	284 ± 174	<0.001
Eosinophils (/μL)	154 ± 156	58 ± 72	<0.001
Basophils (/μL)	22 ± 30	20 ± 21	0.82
Platelets (× 10 ³ /mL)	248 ± 101	194 ± 100	0.018
TP (g/dL)	7.1 ± 0.5	5.9 ± 0.9	<0.001
AST (U/L)	24 ± 10	73 ± 88	<0.001
ALT (U/L)	28 ± 21	43 ± 24	<0.001
LDH (U/L)	206 ± 56	453 ± 223	<0.001
Cre (mg/dL)	0.72 ± 0.20	0.78 ± 0.38	0.596
CRP (mg/dL)	1.8 ± 2.5	10.3 ± 7.8	<0.001
SP-A (ng/mL)	57.1 ± 33.7	117.7 ± 60.0	<0.001
SP-D (ng/mL)	61.9 ± 50.7	237 ± 210	<0.001
KL-6 (U/mL)	225 ± 84	396 ± 237	<0.001
IL-6 (pg/mL)	11.3 ± 41.1	246.9 ± 617.3	<0.001
Hypertension (%)	0.09	0.33	0.042
Diabetes Mellitus (%)	0.03	0.08	0.431
CT score (median)	3.6 ± 3.6 (3)	16.4 ± 6.28 (18)	<0.001

Abbreviations: BMI, body mass index; WBC, white blood cells; TP, total protein; AST, alanine aminotransferase; ALT, aspartate aminotransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein; SP-A, surfactant protein A; SP-D, surfactant protein D; KL-6 Krebs von den Lungen-6

Table 2. Correlation between chest CT score and laboratory data determined using Spearman rank correlations (r).

vs Score	ρ	p
CRP	0.8911	<0.001
LDH	0.826	<0.001
IL-6	0.7711	<0.001
AST	0.6794	<0.001
SP-D	0.6268	<0.001
SP-A	0.5996	<0.001
ALT	0.4296	<0.001
WBC	0.4228	<0.001
KL-6	0.3489	<0.001

Figures

Figure 1

Comparison of serum level of (A) SP-A and (B) SP-D between non-severe cases (closed circle), severe cases (closed triangle), and control subjects (opened square). Human SP-A and SP-D levels in serum from healthy volunteers and COVID-19 patients were assessed by ELISA as described in the Methods section. Data are expressed as the mean $\pm$ SD. Differences between groups were analyzed by non-repeated ANOVA with the Student–Newman–Keuls test. * $p < 0.05$; ** $p < 0.01$

Figure 2

Receiver operating characteristic (ROC) curves of the serum biomarkers SP-A, SP-D, KL-6, LDH, CRP, and IL-6 with respect to the severity of COVID-19. AUC, area under the curve.

Figure 3

Correlation between chest CT score and serum level of SP-A, SP-D, KL-6, and LDH as determined by Spearman rank correlations (ρ), linear regression analysis, and 95% confidence intervals. ** $p < 0.01$

Figure 4

Receiver operating characteristic (ROC) curves of the serum biomarkers SP-A, SP-D, KL-6, LDH, CRP, and IL-6 with respect to chest CT score. AUC, area under the curve.

Figure 5

A typical case of COVID-19 progression in which serum levels of SP-A and SP-D were successively monitored.