

The Significance of KL-6 as Prognosis Monitoring Biomarker in Patients With Severe COVID-19 From Stabilized Stage Toward Convalescence

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Abstract

Background: This study aims to identify some biomarkers for monitoring the recovery of lung injury in severe COVID-19 patients from stabilized stage toward convalescence.

Methods: We enrolled participants who diagnosed with severe COVID-19 (n = 28) and health volunteers (n = 25) from Taikang Tongji (Wuhan) Hospital. The patients were in a stabilized stage and had a course of 48.1 ± 12.8 days. We followed these patients for 90 days. The blood routine, cytokines (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17A, TNF- α , IFN- α , IFN- γ), type II alveolar epithelium injury indicators (Surfactant protein A (SP-A), Krebs von den Lungen-6 (KL-6)) and chest CT were tested on the 1, 30, 60, and 90 days after enrollment.

Results: In stabilized stage, the parameters of blood routine and some cytokines (IL-1 β , IL-2, IL-4, IL-12p70, TNF- α) had bounced back to normal ($p > 0.05$). Some cytokines (IL-5, IL-6, IL-10, IL-17A, IFN- α , IFN- γ) and type II alveolar epithelium injury indicators (SP-A and KL-6) were still higher than normal ($p < 0.05$). During the stabilized stage to convalescence, in spite of the variation of monocyte count, monocyte/lymphocyte ratio, IL-5, IL-10, IL-12p70, IL-17A, IFN- γ , IFN- α , SP-A and KL-6 were downward trend ($p < 0.05$), only KL-6 level ($p < 0.05$) could simultaneously reflect the lung injury volume which be measured by CT.

Conclusions: Our preliminary data indicated that KL-6 could be an effective prognostic biomarker for monitoring the recovery of lung function in patients with severe COVID-19 from stabilized stage toward convalescence.

Background

Severe acute respiratory syndrome CoV-2 (SARS-CoV-2) caused the corona virus disease 2019 (COVID-19) and has become a public health emergency of international concern. Previous studies on the emergence of COVID-19 and its clinical features suggested that older age, high BMI index, male gender, neutrophil-to-lymphocyte ratio, decreased lymphocyte and some indicators (such as D-dimer, IL-6 and C-reactive protein (CRP)) elevated at initial stage or progressive stage might be the risk factors for developing into severe type of COVID-19 [1, 2]. However, in patients with severe COVID-19 from stabilize to convalescent stage, there has been no literature report on which indicators maybe related to lung injury repair.

As known that type II alveolar epithelial cells are the key cells for maintaining lung structure and function [3], meanwhile its expressed angiotensin-converting enzyme 2 (ACE2) which is the main receptor of SARS-COV-2 virus [4]. Therefore, COVID-19 lung injury is mainly caused by damage to type II alveolar epithelium. Alveolar type II alveolar epithelium mainly uses pulmonary surfactant to maintain the normal structure and function of the lung [5]. Among them, the most studied pulmonary surfactants are surfactant protein A (SP-A) [6-8] and Krebs von den Lungen-6 (KL-6) [9, 10]. There is evidence that SP-A secreted by type II alveolar cells increases during infection [11]. These protein are responsible for

recognizing and conditioning microorganisms and presenting them to immune cells, such as alveolar macrophages, to enhance microbial and virus clearance [12]. KL-6 is a high-molecular-weight glycoprotein expressed on the extracellular surface of type II alveolar cells and bronchial epithelial cells [13]. KL-6 is elevated in serum of patients with interstitial lung diseases (ILDs), such as idiopathic pulmonary fibrosis and hypersensitivity pneumonitis [14, 15]. It is mainly produced by damaged or regenerating alveolar type II alveolar cells [16].

This preliminary study analyzed the variation of serum concentrations of type II alveolar epithelial injury indicators (SP-A and KL-6) in severe COVID-19 patients from stabilize to convalescent stage, and to verify their potential as the monitoring indicators for COVID-19 lung injury rehabilitation.

Materials And Methods

Participants

In this study, we enrolled participants (n = 28) who diagnosed with severe COVID-19 and health volunteers (n = 25) from Taikang Tongji (Wuhan) Hospital. The patients with severe COVID-19 had a course of 48.1 ± 12.8 days and were in a stabilized stage when enrolled. We followed these patients for 90 days (day 1, 30, 60 and 90 after enrollment). All participants gave written informed consent and the study was approved by the Ethics Review Board at Tongji University. All the patients had given their informed consent.

Blood routine tests

Blood was collected via venipuncture of an antecubital vein into one 5 mL K₂EDTA (BD Vacutainer) tube and measured immediately by measured by commercially available assay kits according to the manufacturers' protocols (XN2000, Sysmex, Japan).

Serum collection

Whole blood (5 mL) was collected from patients and healthy controls at enrollment (day 1), and on 30, 60 and 90 days. The sample was centrifuged at 3,000 r/min for 10 min and the serum was sub-packed and stored at -80 °C.

Cytokines assay

Cytokine combination detection kit (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17A, TNF- α , IFN- α , IFN- γ) (20192400359, Cell-Genesys, Nanchang, China) was used to detecting serum cytokines. Briefly, 25ul microsphere mixture was added in the test tube, then 25ul serum was added, and mixed thoroughly. Following, 25ul fluorescence detection reagent was added, mixed thoroughly, and incubated for 3 hours. Then 1ml PBS was added for washing, and 200g centrifuged for 5 minutes to remove supernatant, and 100ul of PBS was added for blending. Finally, the sample was detected by BD FACS Calibur (Becton, Dickinson and Company, New Jersey, USA)

ELISA assay

SP-A levels were measured using a commercial ELISA kit (OKAN05694, Aviva Systems Biology, San Diego, USA) as previously described [17].

KL-6 assay

Serum KL-6 concentration (U/ml) was measured with chemiluminescent enzyme immunoassay method (LUMIPULSE G2100, FUJIREBIO INC, Japan) in the study population.

CT image acquisition and imaging data analysis

Chest CT images were obtained by using ONE scanners: SIEMENS SOMATOM go.Top&ALL. A tube voltage of 130kV and automatic tube current modulation (100-400mA) were used. Images were reconstructed with a slice thickness of 1.0mm and an interval of 1.0mm, respectively. Using the pneumonia post-processing software developed by uAI Discover-2019 nCoV (Shanghai United Imaging Intelligence Healthcare Co.,Ltd.) and making quantitative analysis. This software provides automatic segmentation and quantitative analysis of lung lobes. Automatically segment the lung and extract the lung infection area from the thin chest CT image. The baseline and follow-up CT focus volumes of each patient were recorded and compared by two doctors with more than 5 years of experience in chest imaging diagnosis.

Statistical analysis

A p-value less than 0.05 was considered statistically significant. Statistical analysis and graphic representation of the data was performed with SPSS 22.0 and GraphPad Prism 8.0 software.

Results

Severe COVID-19 patient characteristics in stabilized stage

A total of 28 patients with confirmed severe COVID-19 and 25 healthy volunteers participated in this study. As shown in Table 1, there was no statistical difference in age between the two groups (64.93 ± 1.63 vs 64.56 ± 1.55 , $p=0.872$) and gender (male/female, 16 /12 vs 14/11, $p=0.935$). In the parameters of blood routine, white blood cell (WBC) count (6.98 ± 0.53 vs 5.81 ± 0.34 , $p=0.067$), lymphocyte count (2.09 ± 0.30 vs 1.66 ± 0.13 , $p=0.172$), neutrophil count (3.91 ± 0.34 vs 3.41 ± 0.25 , $p=0.244$), monocyte count (0.65 ± 0.06 vs 0.56 ± 0.06 , $p=0.352$), neutrophil/lymphocyte ratio (1.82 ± 0.34 vs 2.34 ± 0.24 , $p=0.255$), monocyte/lymphocyte ratio (0.28 ± 0.05 vs 0.35 ± 0.025 , $p=0.157$) had no statistical difference between two group. Among the cytokines, there were no significant statistical difference in IL-1 β (9.10 ± 3.28 vs 4.65 ± 4.10 , $p=0.411$), IL-2 (2.96 ± 1.04 vs 1.18 ± 1.11 , $p=0.279$), IL-4 (4.97 ± 1.60 vs 1.69 ± 1.51 , $p=0.184$), IL-12p70 (5.04 ± 2.22 vs 0.13 ± 0.11 , $p=0.110$) and TNF- α (9.83 ± 3.81 vs 3.08 ± 2.89 , $p=0.162$). But, there were significant statistical difference in IL-5 (1.72 ± 0.40 vs 0.13 ± 0.10 , $p<0.01$), IL-6 (23.93 ± 4.89 vs 3.78 ± 1.49 , $p<0.01$), IL-10 (9.74 ± 1.75 vs 1.50 ± 0.19 , $p<0.01$), IL-17A (24.94 ± 4.39 vs 2.90 ± 1.03 , $p<0.01$), IFN- γ

(6.65 ± 1.76 vs 0.55 ± 0.22 , $p < 0.01$) and IFN- α (17.71 ± 4.92 vs 1.70 ± 1.55 , $p < 0.01$). At the same time, we detected the indicators of type II alveolar epithelial injury and found that SP-A (247.04 ± 22.45 vs 17.83 ± 8.01 , $p < 0.01$) and KL-6 (977.39 ± 136.93 vs 251.96 ± 20.63 , $p < 0.01$) were significant higher in COVID-19 patients.

ROC analysis of significant indicators in stabilized stage of severe COVID-19 patients

ROC analysis was performed to test the prediction power of the signature of severe COVID-19 in stabilized stage. ROC analysis distinguished these two groups with an area under the curve of IL-5 (AUC_{Signature}=0.938, Figure 1A), IL-6 (AUC_{Signature}=0.934, Figure 1B), IL-10 (AUC_{Signature}=0.967, Figure 1C), IL-17A (AUC_{Signature}=0.791, Figure 1D), IFN- γ (AUC_{Signature}=0.917, Figure 1E), IFN- α (AUC_{Signature}=0.947, Figure 1F), SP-A (AUC_{Signature}=0.989, Figure 1G), KL-6 (AUC_{Signature}=0.931, Figure 1H).

The blood indexes variation of severe COVID-19 patients from stabilized stage toward convalescence

From the above results, we know that during the stabilized stage of severe COVID-19 patients, the parameters of blood routine and some cytokines (IL-1 β , IL-2, IL-4, IL-12p70 and TNF- α) returned to normal, but some cytokines (IL-5, IL-6, IL-10, IL-17A, IFN- γ and IFN- α) and type II alveolar epithelial injury indicators (SP-A and KL-6) were still higher than healthy control group. Therefore, we continuously monitored the blood indexes of severe COVID-19 patients from stabilized stage to convalescence (day 1, 30, 60 and 90 of experiment enrollment). The variation of WBC count ($F=0.789$, $p=0.453$) (Figure 2A), lymphocyte count ($F=0.599$, $p=0.618$) (Figure 2B), neutrophil count ($F=0.974$, $p=0.389$) (Figure 2C), neutrophil/lymphocyte ratio ($F=3.015$, $p=0.055$) (Figure 2E), IL-1 β ($F=2.729$, $p=0.109$) (Figure 2G), IL-2 ($F=3.568$, $p=0.071$) (Figure 2H), IL-4 ($F=2.004$, $p=0.172$) (Figure 2I), IL-6 ($F=0.594$, $p=0.477$) (Figure 2K) and TNF- α ($F=1.627$, $p=0.217$) (Figure 2Q) were not statistically significant. The monocyte count ($F=51.081$, $p < 0.01$) (Figure 2D), monocyte/lymphocyte ratio ($F=40.715$, $p < 0.01$) (Figure 2F), IL-5 ($F=12.571$, $p < 0.01$) (Figure 2J), IL-10 ($F=16.943$, $p < 0.01$) (Figure 2L), IL-12p70 ($F=9.015$, $p < 0.01$) (Figure 2M), IL-17A ($F=4.402$, $p=0.029$) (Figure 2N), IFN- γ ($F=7.288$, $p=0.005$) (Figure 2O), IFN- α ($F=5.928$, $p=0.021$) (Figure 2P), SP-A ($F=13.710$, $p < 0.01$) (Figure 2R) and KL-6 ($F=13.168$, $p < 0.01$) (Figure 2S) were downward trend and the variation were statistically significant.

Serum KL-6 level reflect the severity of lung injury in severe COVID-19 patients from stabilized stage toward convalescence

The COVID-19 patients group were divided into lung injury range $> 1000 \text{ mm}^3$ group ($n=10$) and lung injury range $< 1000 \text{ mm}^3$ group ($n=18$) based on the CT results of day 1 enrollment. Following, we continuously monitored the blood indexes from stabilized stage to convalescence (day 1, 30, 60 and 90 of experiment enrollment). Our data showed that the WBC count ($F=0.301$, $p=0.589$) (Figure 3A), lymphocyte count ($F=4.054$, $p=0.055$) (Figure 3B), neutrophil count ($F=1.274$, $p=0.270$) (Figure 3C), monocytes count ($F=0.021$, $p=0.887$) (Figure 3D), neutrophil/lymphocyte ratio ($F=3.440$, $p=0.076$) (Figure 3E), monocyte/lymphocyte ratio ($F=3.670$, $p=0.067$) (Figure 3F), IL- β ($F=0.221$, $p=0.644$) (Figure 3G), IL-2

(F=0.299, p=0.638) (Figure 3H), IL-4 (F=0.383, p=0.543) (Figure 3I), IL-5 (F=0.827, p=0.374) (Figure 3J), IL-6 (F=0.159, p=0.694) (Figure 3K), IL-10 (F=0.449, p=0.511) (Figure 3L), IL-12p70 (F=0.809, p=0.380) (Figure 3M), IL-17A (F=1.828, p=0.192) (Figure 3N), IFN- γ (F=0.721, p=0.406) (Figure 3O), IFN- α (F=0.239, p=0.630) (Figure 3P), TNF- α (F=0.290, p=0.597) (Figure 3Q) and SP-A (F=0.005, p=0.942) (Figure 3R) were not statistically significant between two groups. Meanwhile, we found that the serum KL-6 (F=5.824, p=0.023) (Figure 3S) was statistically significant between the two groups, and KL-6 was higher in lung injury range >1000 mm³ group than lung injury range <1000 mm³ group. Above data implied that KL-6 maybe serve as a good indicator for monitoring lung injury repair in severe COVID-19 patient from stabilized stage toward convalescence.

The clinical characteristics of a severe COVID-19 patient case from stabilized stage toward convalescence

We selected a 57-year-old female patient confirmed severe COVID-19. Figures 4A to 4D showed the CT imaging results of this patient at different time points (day 1, 30, 60 and 90 of experiment enrollment). Figure 4E was the variation trend of CT results, it showed that the lung injury range decreased along time. Figure 4F and 4G respectively showed the variation of serum SP-A and KL-6 levels at the time points corresponding to the CT results. Combining the results of CT and serology, we found that as the extent of lung injury shrinks, the levels of SP-A and KL-6 gradually decrease. In addition, the variation of parameters of blood routine and cytokines did not match with the CT results, and the data not show.

Discussion

The COVID-19 virus has been shown to invade human alveolar type II alveolar epithelium through mainly ACE2 and induce the lung injury [18]. It is reported that alveolar type II alveolar epithelium are the key cells for maintaining the normal structure and function of the lung, and it is mainly through pulmonary surfactant (SP-A, KL-6 and so on) [3, 5]. Both SP-A and KL-6 are produced by type II alveolar epithelial cells, which proliferate following lung injury. Therefore, they have been extensively studied as the biomarkers of lung injury [19].

Most of the SP-A found in the alveolar space is bound to dipalmitoyl phosphatidylcholine (DPPC), which is the main component of pulmonary surfactant. Meanwhile, SP-A could interact with alveolar macrophages (AM), and regulates many of its functions under infection and oxidative stress condition for participating in congenital lung defense and non-antibody-mediated immune response [20]. KL-6 is a high molecular weight human transmembrane mucin 1 and promotes the migration and proliferation of lung fibroblasts [21]. The serum level of KL-6 was capable of discriminating between ILD and other common lung diseases. A cutoff of 500 U/ml was established to distinguish patients with ILD from healthy controls and those with other lung disorders. Therefore, KL-6 may be an effective biomarker for ILD diagnosis and monitoring [22]. It is also reported that the serum concentrations of KL-6 may be a prognostic biomarker of COVID-19 severity in the stage of disease initiation and progression [9]. However,

it is not clear whether SP-A and KL-6 could be used as indicators for monitoring lung injury repair in patients with severe COVID patients from stabilize to convalescent stage.

In this study, we analyzed the variation of type II alveolar epithelial injury indicators (SP-A and KL-6), the parameters of blood routine and cytokines (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17A, TNF- α , IFN- α , IFN- γ) in patients with severe COVID patients from stabilize to convalescent stage for the first time.

In stabilized stage, the parameters of blood routine and some cytokines (IL-1 β , IL-2, IL-4, IL-12p70, TNF- α) had bounced back to normal. Some cytokines (IL-5, IL-6, IL-10, IL-17A, IFN- α , IFN- γ) and type II alveolar epithelium injury indicators (SP-A and KL-6) were still higher than normal. This suggests that some of the indicators that are meaningful for the initial and progression stages of COVID-19 may not be appropriate for disease surveillance in stabilized stage.

Following, we continuously monitored the blood indexes of severe COVID-19 patients from stabilized stage to convalescence (day 1, 30, 60 and 90 of experiment enrollment). Despite the variation of monocyte count, monocyte/lymphocyte ratio, IL-5, IL-10, IL-12p70, IL-17A, IFN- γ , IFN- α , SP-A and KL-6 were downward trend, only KL-6 level could simultaneously reflect the lung injury volume. Thus, our data implied that KL-6 maybe serve as a good indicator for monitoring lung injury repair in severe COVID-19 patient from stabilized stage toward convalescence, especially for some primary medical settings where CT imaging is not available.

Meanwhile, we selected a 57-year-old female patient with severe COVID-19 pneumonia, and we dynamically followed up the CT imaging changes in the lung of this patient, and also detected SP-A, KL-6, blood routine and cytokines. We found that the patient had gone from the stable stage of the disease to the stage of rehabilitation, among the measured hematological indicators, only the changes of SP-A and KL-6 were corresponding to the changes of pulmonary CT, suggesting that SP-A and KL-6 could well imply the outcome of the disease. At the same time, we found that the KL-6 level of the patient had basically returned to normal when the endpoint of observation was reached, and there was no statistical difference compared with the normal group, but SP-A was still significantly higher than normal. Whether this indicates persistent lung injury or a normal pattern of lung injury recovery in patient with severe COVID-19 pneumonia, it requires more clinical research.

Conclusion

In conclusion, our preliminary data indicate that serum type II alveolar epithelium injury indicators, especially KL-6 could be useful markers for monitoring and evaluating the recovery of lung injury in severe COVID-19 patients from stabilized stage toward convalescence. This hypothesis will need to be further tested in the future.

Abbreviations

SARS-CoV-2: severe acute respiratory syndrome CoV-2; COVID-19: the corona virus disease 2019; CRP: C-reactive protein; ACE2: angiotensin-converting enzyme 2; SP-A: pulmonary surfactants are surfactant protein A; KL-6: Krebs von den Lungen-6; ILDs: interstitial lung diseases; DPPC: dipalmitoyl phosphatidylcholine.

Declarations

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Availability of data and materials

The datasets, as well as the patent materials, used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LZM is the guarantor of this research. LZM, FLY and LQ were responsible for study concept and design. HL, LL, ZM, ZH, JEP contributed to patient recruitment and patient follow-up. ZL was responsible for outcome measurement. WL, CNZ, YJY finished data acquisition. HL finished data analysis. All authors contributed to the drafting of this manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Prior to its start, the study protocol was in accordance with the Declaration of Helsinki, and approved by the ethics committee of East Hospital, Tongji University, Shanghai, China. The reference number for the study is 2019-TJDX-81. Informed consents about the study procedures were signed and obtained from all the subjects before the trial.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interest

References

1. Wang L: **C-reactive protein levels in the early stage of COVID-19.** *Med Mal Infect* 2020, **50**:332-334.
2. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A: **The role of biomarkers in diagnosis of COVID-19 - A systematic review.** *Life Sci* 2020, **254**:117788.
3. Traver G, Mont S, Gius D, Lawson WE, Ding GX, Sekhar KR, Freeman ML: **Loss of Nrf2 promotes alveolar type 2 cell loss in irradiated, fibrotic lung.** *Free Radic Biol Med* 2017, **112**:578-586.
4. Ziegler CGK, Allon SJ, Nyquist SK, Mbanjo IM, Miao VN, Tzouanas CN, Cao Y, Yousif AS, Bals J, Hauser BM, et al: **SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues.** *Cell* 2020, **181**:1016-1035 e1019.
5. Martinez-Calle M, Olmeda B, Dietl P, Frick M, Perez-Gil J: **Pulmonary surfactant protein SP-B promotes exocytosis of lamellar bodies in alveolar type II cells.** *FASEB J* 2018, **32**:4600-4611.
6. Fandino J, Vaz AA, Toba L, Romani-Perez M, Gonzalez-Matias L, Mallo F, Diz-Chaves Y: **Liraglutide Enhances the Activity of the ACE-2/Ang(1-7)/Mas Receptor Pathway in Lungs of Male Pups from Food-Restricted Mothers and Prevents the Reduction of SP-A.** *Int J Endocrinol* 2018, **2018**:6920620.
7. Zhu T, Li C, Zhang X, Ye C, Tang S, Zhang W, Sun J, Huang N, Wen F, Wang D, et al: **GLP-1 Analogue Liraglutide Enhances SP-A Expression in LPS-Induced Acute Lung Injury through the TTF-1 Signaling Pathway.** *Mediators Inflamm* 2018, **2018**:3601454.
8. Samukawa T, Hamada T, Uto H, Yanagi M, Tsukuya G, Nosaki T, Maeda M, Hirano T, Tsubouchi H, Inoue H: **The elevation of serum napsin A in idiopathic pulmonary fibrosis, compared with KL-6, surfactant protein-A and surfactant protein-D.** *BMC Pulm Med* 2012, **12**:55.
9. d'Alessandro M, Cameli P, Refini RM, Bergantini L, Alonzi V, Lanzarone N, Bennett D, Rana GD, Montagnani F, Scolletta S, et al: **Serum KL-6 concentrations as a novel biomarker of severe COVID-19.** *J Med Virol* 2020.
10. d'Alessandro M, Bergantini L, Cameli P, Lanzarone N, Antonietta Mazzei M, Alonzi V, Sestini P, Bargagli E: **Serum KL-6 levels in pulmonary Langerhans' cell histiocytosis.** *Eur J Clin Invest* 2020:e13242.
11. Mouhieddine-Gueddiche OB, Pinteaur C, Chailley-Heu B, Barlier-Mur AM, Clement A, Bourbon JR: **Dexamethasone potentiates keratinocyte growth factor-stimulated SP-A and SP-B gene expression in alveolar epithelial cells.** *Pediatr Res* 2003, **53**:231-239.
12. Thorenoor N, Kawasaki YI, Gandhi CK, Floros J: **Sex-Specific Regulation of Gene Expression Networks by Surfactant Protein A (SP-A) Variants in Alveolar Macrophages in Response to Klebsiella pneumoniae.** *Front Immunol* 2020, **11**:1290.
13. Kondo T, Hattori N, Ishikawa N, Murai H, Haruta Y, Hirohashi N, Tanigawa K, Kohno N: **KL-6 concentration in pulmonary epithelial lining fluid is a useful prognostic indicator in patients with acute respiratory distress syndrome.** *Respir Res* 2011, **12**:32.
14. Lee JS, Lee EY, Ha YJ, Kang EH, Lee YJ, Song YW: **Serum KL-6 levels reflect the severity of interstitial lung disease associated with connective tissue disease.** *Arthritis Res Ther* 2019, **21**:58.

15. Bergantini L, Bargagli E, Cameli P, Cekorja B, Lanzarone N, Pianigiani L, Vietri L, Bennett D, Sestini P, Rottoli P: **Serial KL-6 analysis in patients with idiopathic pulmonary fibrosis treated with nintedanib.** *Respir Investig* 2019, **57**:290-291.
16. d'Alessandro M, Bergantini L, Cameli P, Vietri L, Lanzarone N, Alonzi V, Pieroni M, R MR, Sestini P, Bonella F, Bargagli E: **Krebs von den Lungen-6 as a biomarker for disease severity assessment in interstitial lung disease: a comprehensive review.** *Biomark Med* 2020, **14**:665-674.
17. He L, Sun F, Wang Y, Zhu J, Fang J, Zhang S, Yu Q, Gong Q, Ren B, Xiang X, et al: **HMGB1 exacerbates bronchiolitis obliterans syndrome via RAGE/NF-kappaB/HPSE signaling to enhance latent TGF-beta release from ECM.** *Am J Transl Res* 2016, **8**:1971-1984.
18. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, et al: **A pneumonia outbreak associated with a new coronavirus of probable bat origin.** *Nature* 2020, **579**:270-273.
19. Kambe M, Ohshimo S, Kohno N: **[Immunity tests for respiratory diseases–SP-A, SP-D, KL-6].** *Rinsho Byori* 2007, **55**:381-387.
20. Kishore U, Greenhough TJ, Waters P, Shrive AK, Ghai R, Kamran MF, Bernal AL, Reid KB, Madan T, Chakraborty T: **Surfactant proteins SP-A and SP-D: structure, function and receptors.** *Mol Immunol* 2006, **43**:1293-1315.
21. Hirasawa Y, Kohno N, Yokoyama A, Inoue Y, Abe M, Hiwada K: **KL-6, a human MUC1 mucin, is chemotactic for human fibroblasts.** *Am J Respir Cell Mol Biol* 1997, **17**:501-507.
22. Kobayashi J, Itoh Y, Kitamura S, Kawai T: **[Establishment of reference intervals and cut-off value by an enzyme immunoassay for KL-6 antigen, a new marker for interstitial pneumonia].** *Rinsho Byori* 1996, **44**:653-658.

Table

Table 1.

Participant characteristics. The main characteristics of population including, age, gender, the parameters of blood routine, cytokines, and type II alveolar epithelium injury indicators (SP-A and KL-6) at the admission (day 1).

Parameters (Mean±SEM)	Severe COVID-19 patients(n=28)	Healthy control(n=25)	p value
Age	64.93±1.63	64.56±1.55	0.872
Gender (Male/Female)	16/12	14/11	0.935
WBC (10 ⁹ /L)	6.98±0.53	5.81±0.34	0.067
Lymphocyte (10 ⁹ /L)	2.09±0.30	1.66±0.13	0.172
Neutrophil (10 ⁹ /L)	3.91±0.34	3.41±0.25	0.244
Monocyte (10 ⁹ /L)	0.65±0.06	0.56±0.06	0.352
Neutrophil/Lymphocyte	1.82±0.34	2.34±0.24	0.255
Monocyte/Lymphocyte	0.28±0.05	0.35±0.025	0.157
IL-1β(pg/ml)	9.10±3.28	4.65±4.10	0.411
IL-2(pg/ml)	2.96±1.04	1.18±1.11	0.279
IL-4(pg/ml)	4.97±1.60	1.69±1.51	0.184
IL-5(pg/ml)	1.72±0.40	0.13±0.10	<0.01
IL-6 (pg/ml)	23.93±4.89	3.78±1.49	<0.01
IL-10(pg/ml)	9.74±1.75	1.50±0.19	<0.01
IL-12p70(pg/ml)	5.04±2.22	0.13±0.11	0.110
IL-17A(pg/ml)	24.94±4.39	2.90±1.03	<0.01
IFN-γ(pg/ml)	6.65±1.76	0.55±0.22	<0.01
IFN-α(pg/ml)	17.71±4.92	1.70±1.55	<0.01
TNF-α(pg/ml)	9.83±3.81	3.08±2.89	0.162
SP-A (ng/ml)	247.04±22.45	17.83±8.01	<0.01
KL-6 (U/ml)	977.39±136.93	251.96±20.63	<0.01

Figures

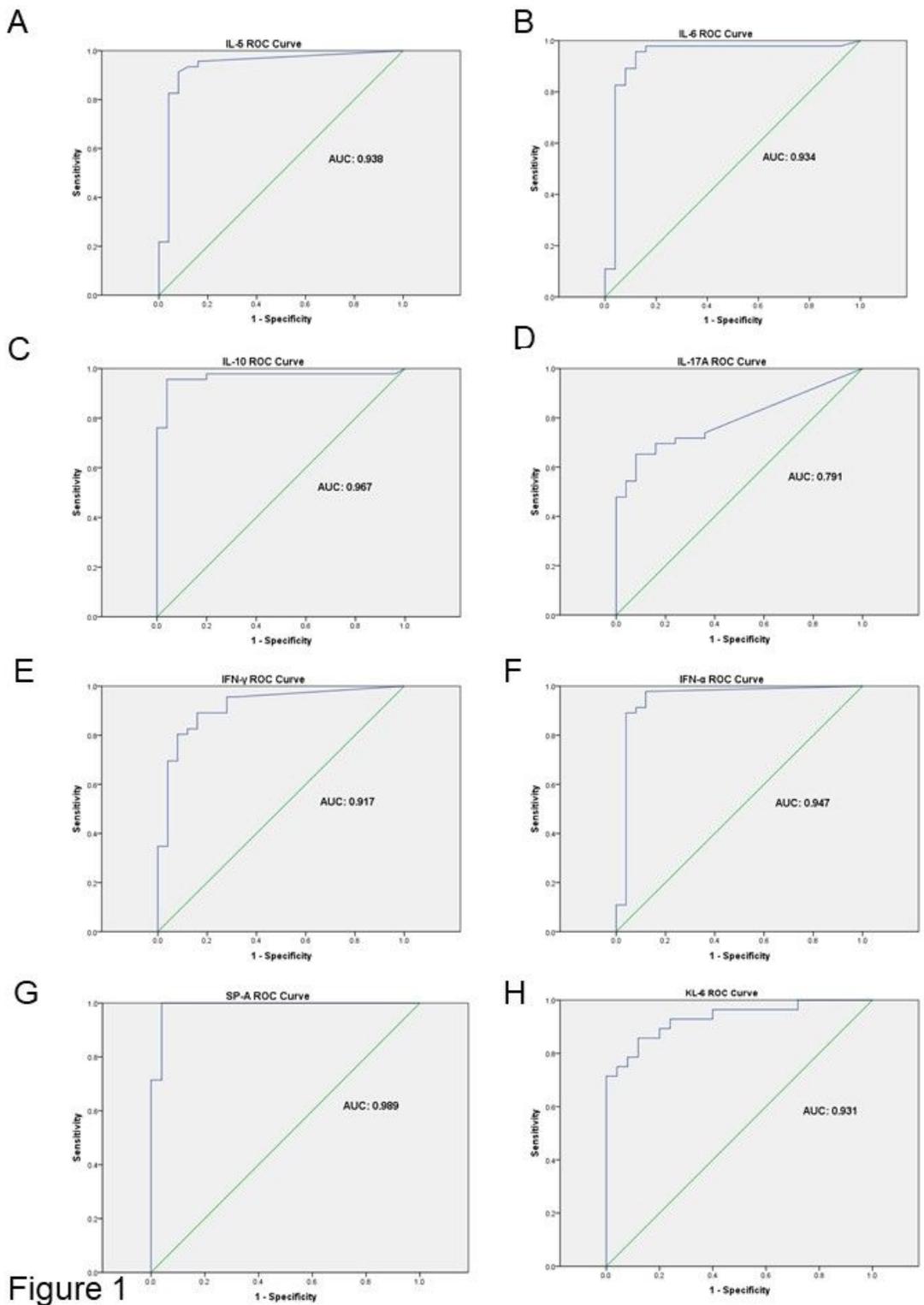


Figure 1

Figure 1

ROC curve analysis of different serum biomarkers. Figure A-H respectively shows IL-5, IL-6, IL-10, IL-17A, IFN- γ , IFN- α , SP-A and KL-6 level as the diagnostic marker of severe COVID-19 in stabilized stage.

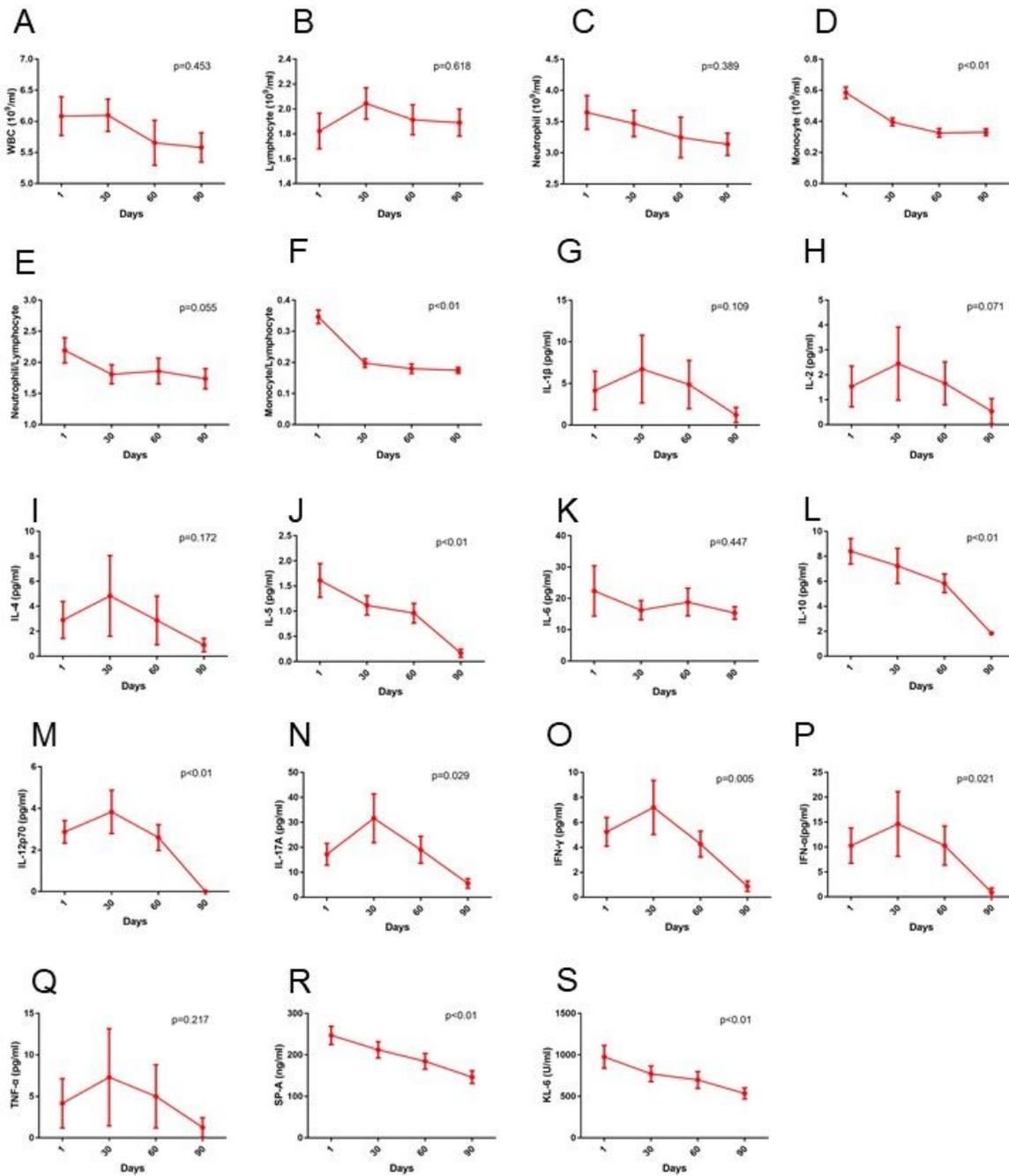


Figure 2

Figure 2

Correlation between altered serum indicators levels and lung injury recovery in patients with severe COVID-19 from stabilized stage toward convalescence. Figure A-S respectively shows the trend of blood WBC count, lymphocyte count, neutrophil count, monocyte count, neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio, IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17A, IFN- γ , IFN- α , TNF- α , SP-A and

KL-6 at 1, 30, 60, 90 day after admission in patients with severe COVID-19 from stabilized stage toward convalescence.

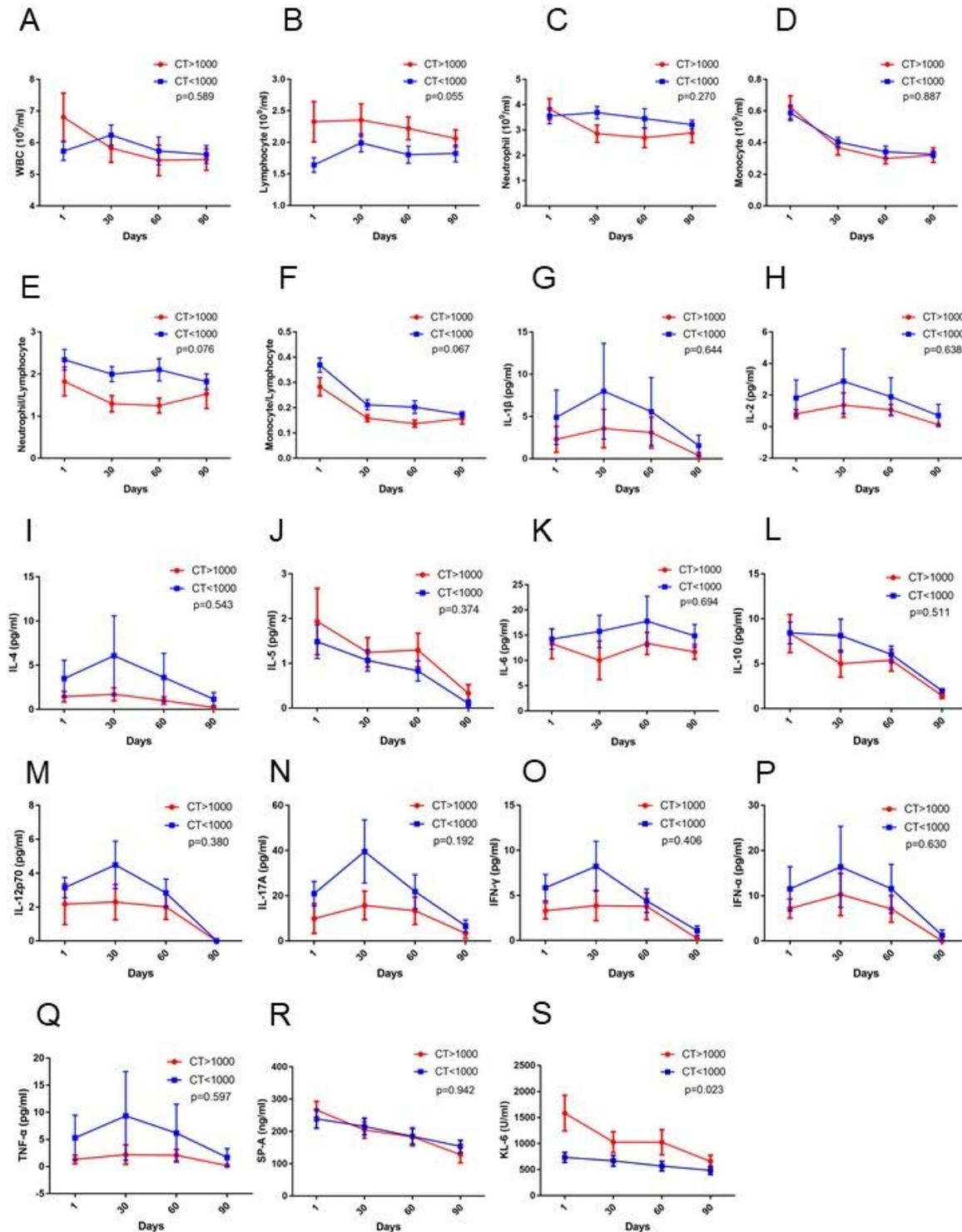


Figure 3

Figure 3

Correlation between altered serum indicators levels and degree of lung injury in patients with severe COVID-19 from stabilized stage toward convalescence. Figure A-S respectively shows the trend of blood WBC count, lymphocyte count, neutrophil count, monocyte count, neutrophil/lymphocyte ratio,

monocyte/lymphocyte ratio, IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17A, IFN- γ , IFN- α , TNF- α , SP-A and KL-6 at 1, 30, 60, 90 day after admission in patients with severe COVID-19 from stabilized stage toward convalescence. The red line stand for lung injury volume greater than 1000 mm³ group, and the blue line stand for lung injury volume less than 1000 mm³ group.

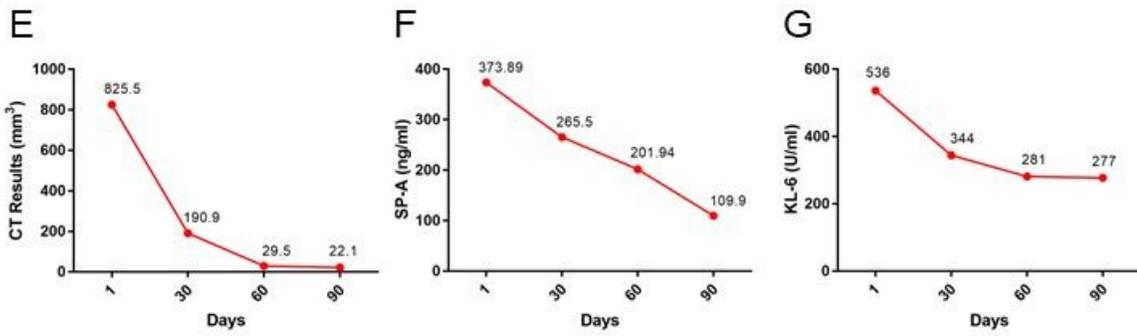
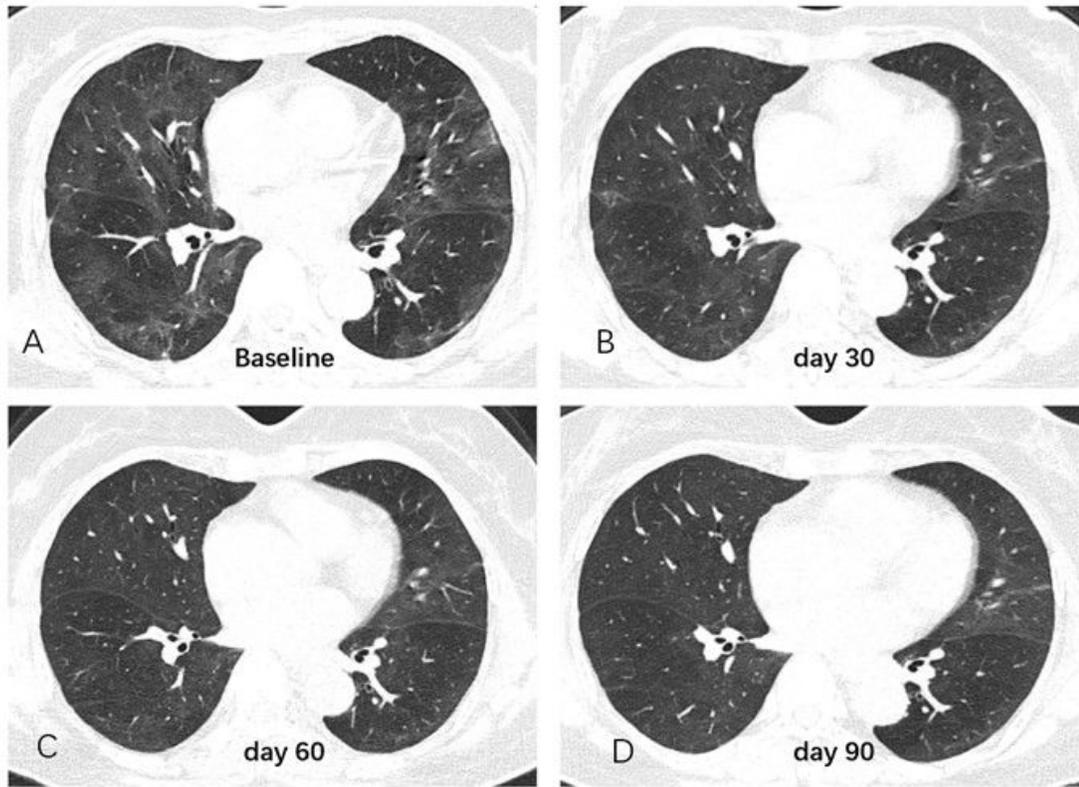


Figure 4

Figure 4

The clinical characteristics of a severe COVID-19 patient case from stabilized stage toward convalescence. A-D shows sequence CT scan of 57-year-old female diagnosed with severe COVID-19 (baseline, D30, D60, D90). Figure A shows a flake ground glass shadow in both lungs. Figure B shows that the range of ground glass shadow has been reduced, and there is a residual shadow of fiber strips. Figure C-D shows ground glass shadow is basically absorbed. Figure 4E was the variation trend of CT results. Figure 4F and 4G respectively showed the variation of serum SP-A and KL-6 levels.