TGF-β and CCL18 as indicators for predicting and monitoring the development of pulmonary fibrosis in patients with COVID-19

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Abstract

Background

Many COVID-19 patients have been discharged, but lung injury, including pulmonary fibrosis, might lead to long-term impairment. This study aimed to evaluate predictors and monitors of pulmonary fibrosis in patients with COVID-19.

Methods

Thirty-five convalescent patients with severe COVID-19, after appropriate medical treatments, were recruited. According to evidence of fibrosis on initial computed tomography (CT), the patients were divided into mild-to-moderate and severe groups. Levels of transforming growth factor beta (TGF-β), chemokine ligand 18 (CCL18), type III procollagen peptide (P□P), hyaluronic acid (HA), laminin (LN), and type IV collagen (C□) were determined. Laboratory tests, clinical data, and CT features at different stages were collected and analyzed, and the prognostic performance of these parameters was evaluated.

Results

Severe fibrosis was found in 76.29% (26/35) of patients. However, most baseline laboratory characteristics were normal. Fibrosis indicators (TGF-β: 66.67 ± 158.57 vs 55.84 ± 126.43 pg/mL, P = 0.006; CCL18: 364.27 ± 167.70 vs 84.47 ± 60.67 ng/mL, P = 0.000; P□P: 54.12 ± 55.34 vs 17.15 ± 2.48 ng/mL, P = 0.000; HA: 122.47 ± 78.84 vs 59.74 ± 18.01 ng/mL, p = 0.000; LN: 55.43 ± 46.44 vs 24.25 ± 7.79 ng/mL, P = 0.000; C□: 24.77 ± 14.97 vs 15.32 ± 1.15 ng/mL, P = 0.001) were elevated in patients compared with controls. Over 90 days' follow-up, HRCT scores gradually decreased from 22.48 ± 16.13 to 10.33 ± 11.11 (P < 0.001), and mMRC scores decreased from 3.27 ± 0.32 to 1.48 ± 0.33, and all fibrosis indicators, except for P□P, gradually declined with the improvement of pulmonary fibrosis. Moreover, TGF-β and CCL18 levels were lower in the mild-to-moderate than severe fibrosis group (88.16 ± 97.45 vs 205.93 ± 170.57 pg/mL, P = 0.024; 241.84 ± 125.37 vs 366.64 ± 161.06 ng/mL, P = 0.038), and patients with elevated baseline levels of serum TGF-β and CCL18 had longer rehabilitation times.

Conclusions

TGF-β and CCL18 may be promising biomarkers for predicting and monitoring the development of pulmonary fibrosis in patients with COVID-19.

Background

In December 2019, the first reports emerged of a novel virus in Wuhan, China, now called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belonging to the *Coronaviridae* family and causing respiratory system symptoms, ranging from a mild cold to severe acute respiratory syndrome (SARS)[1, 2]. The disease caused by this virus was declared an epidemic by the World Health Organization (WHO), which was

officially named coronavirus disease 2019 (COVID-19) and confirmed to be a global public health threat. As of 24 July 2020, nearly 16 million people have been infected with this novel coronavirus and about 630 000 people have died worldwide, sweeping across about 150 countries[3].

COVID-19 shows a diverse course, ranging from asymptomatic to respiratory failure. It's reported that about 40% of patients with COVID-19 develop acute respiratory distress syndrome (ARDS), and 20% of them belong to severe cases, a disease that is characterized by rapid-onset fibrosis (respiratory rate > 30/min, SpO_2/FiO_2 < 300, saturation < 93%, and shortness of breath)[4, 5]. According to Zhou et al., only 9 out of 137 COVID-19 patients who survived exhibited ARDS, and a substantial proportion die as a result of developing ARDS with progressive pulmonary fibrosis[6, 7]. Radiological studies have shown that 12.7% of cases have irregular solid nodules and 17.5% of cases have fibrous stripes [8, 9]. In addition, 85% of COVID-19 patients progress, with enlargement of the nodules and stripes, and the majority of patients have patchy ground-glass opacities[10]. These data strongly imply that not only is pulmonary fibrosis one of the major complications of COVID-19, which results in chronic dyspnea and affects the quality of life of patients, but that it may be the cause of mortality in COVID-19.

However, the long-term pulmonary outcomes of COVID-19 remain speculative without appropriate prospective study. There are insufficient evidences on the severity and development of pulmonary fibrosis related to COVID-19, especially for recovered patients. Moreover, it has rarely been reported how to predict the development of the pulmonary fibrosis and how to dynamically monitor it. Currently, high-resolution computed tomography (HRCT) is generally used to diagnosis the pulmonary fibrosis. Dr Yu and colleagues analyzed the CT imaging features of patients with COVID-19 in different stages and found that irregular interface,interstitial thickening, parenchymal band and coarse reticular patterns could predict the development of pulmonary fibrosis[11]. Additionally, serial lung function testing is also generally used to predict and monitor the progression of pulmonary fibrosis[12]. However, the clinical course of COVID-19 is still unclear and highly variable. In addition, HRCT and lung function testing are not convenient or economical, and it is difficult to achieve dynamic monitoring. Therefore, the aim of this study is to follow up patients after treatment for COVID-19, to identify more and better clinical parameters that might contribute to predicting and monitoring the development of pulmonary fibrosis after discharge, and seek possible ways of treatment and prevention.

Materials And Methods

Participants

The study was approved by the Ethics Committee of Tong Ji University (DFSC-2019(CR)-03), and informed consent was obtained from all the subjects prior to the study.

In this study, 35 patients (67.05 ± 9.67 years, 22 males) who had been hospitalized for COVID-19 at the Sixth Affiliated Hospital and Taikang Tongji Hospital from 10 March 2020 to 30 June 2020 were tracked after discharge. Patients in this study were confirmed by a real-time PCR test for SARS-CoV-2 and further treated according to the guidance of the National Health Commission of China (Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (7th Edition)). When the patients were recruited, most of them were in the

convalescence phase and only those defined as severe according to the clinical classification of COVID-19 released by the National Health Commission of China were initially enrolled.

Consenting individuals (n = 60; 65.88 ± 8.24 years, 19 males) were included as the control group. They had no history of novel coronavirus pneumonia and were not on any medication. They had normal chest CT imagining and normal lung function test parameters.

Clinical evaluation and biologic measurements

The patients were assessed through a 90-day observation after entering the study group. Radiological outcomes and clinical laboratory were recorded and certified by a trained group of doctors. The details are displayed in the flow chart of follow-up [see Additional file 1]. All the serum samples used in this study were the leftovers otherwise “discarded” after routine biochemical evaluation in a clinical laboratory. Baseline characteristics, including age, gender, underlying conditions, symptoms, and treatment regimens that might correlate with the clinical manifestations and therapeutic outcomes in COVID-19 patients were also collected and analyzed. The modified Medical Research Council (mMRC) dyspnea scale was used to measure the degree of disability that breathlessness poses on day-to-day activities on a scale from 0 to 4: 0, no breathlessness except on strenuous exercise; 1, shortness of breath when hurrying on the level or walking up a slight hill; 2, walks slower than people of same age on the level because of breathlessness or has to stop to catch breath when walking at their own pace on the level; 3, stops for breath after walking ± 100 m or after few minutes on the level; and 4, too breathless to leave the house, or breathless when dressing or undressing[13].

Computed tomography imaging

CT scanning was performed during hospitalization and at follow-up on Day 30, Day 60, and Day 90. CT images were collected from the 35 study patients. The results were evaluated independently by three radiologists with 6, 12, and 28 years’ imaging experience, blinded to all clinical information. Chest CT imaging was obtained using ONE scanners: Siemens SOMATOM go.Top and SOMATOM go.All. A tube voltage of 130 kV and automatic tube current modulation (100–400 mA) were used. Images were reconstructed with a slice thickness of 1.0 mm and an interval of 0.8 mm.

According to the Fleischer Society CT criteria for lung fibrosis, the distribution and extent of ground-glass opacities, reticular patterns, interlobular septal thickening and airspace consolidation were evaluated, as detailed in past study[14, 15]. A total CT score of 0 was defined as no fibrosis, 1–10 as mild fibrosis, 10–20 as moderate fibrosis and ≥ 20 as severe fibrosis. So the COVID-19 patient group was further divided into severe and mild-to-moderate fibrosis groups.

TGF-β and CCL18 assays

Blood samples were collected from the follow-up patients and control subjects after 12 hours of overnight fasting. After laboratory testing, the remaining serum was kept at − 80°C until TGF-β and CCL18 analysis. Serum concentrations of TGF-β and CCL18 were measured using the corresponding ELISA kit (TGF-β: #OKBB00253, CCL18: #OKCD07465; AVIVA Systems Bio, USA) according to the protocol of the reagent assay. The principle of the assay is that TGF-β/CCL18 antigen in samples binds to its target-specific antibody by antigen–antibody reaction. The absorbance was measured to determine TGF-β and CCL18 concentrations.

Statistical analysis

The normality of the corresponding quantitative data was checked using the Kolmogorov-Smirnov test. The normal distribution data were presented as mean ± standard deviation (SD) and differences between the two groups were assessed using a two independent samples t test.The data of skew distribution were presented as median value and interquartile range (IQR), and differences between the two groups were assessed using Mann–Whitney *U* test. A chi-squared test or Fisher's exact test was used for categorical variables as appropriate. One-way repeated measures ANOVA was applied to evaluate the variables at the different follow-up times. Kaplan-Meier analysis and log-rank Cox analysis were used to evaluate the probability of prognosis .All statistical analyses in the study were performed using SPSS22.0 software (IBM Corp., Armonk, NY, USA)

Results

Baseline clinical symptoms and characteristic of included patients

The average age of patients in the COVID-19 group and control group was 67.02 ± 9.18 and 65.53 ± 8.04 years, respectively. There were no obvious differences between the two groups in the proportion of patients with regard to gender and comorbidities. Clinical conditions and baseline symptoms of the patients with COVID-19 are listed in Tables 1 and 2. In this study, complications mainly included double pneumonia (100%), hepatic injury (4.35%), renal dysfunction (4.35%), and intestinal disease (8.70%). The treatment regimens mainly included antiviral therapy, antibiotic therapy, cellular immunotherapy, glucocorticoid therapy, and Chinese herbs. According to the clinical classification of COVID-19 released by the National Health Commission of China, all patients in this study were considered severe cases. In terms of the pneumonia symptoms, most patients manifested cough (91.30%), phlegm (67.39%), chest distress (100%), fatigue (82.61%), and shortness of breath (80.43%), but no longer exhibited fever (26.08%) as most of them were in the convalescence phase. The clinical characteristics of the mild-to-moderate fibrosis group and severe fibrosis group are comparatively presented in Tables 1 and 2. There were no differences between the groups in complications, treatment regimens, and clinical symptoms.

Table 1
Baseline characteristics of 35 patients with COVID-19 Pneumonia and Control individuals.

Baseline characteristic	COVID-19 group				Control group	P^2
	Mild to moderate-fibrosis group	Severe-fibrosis group	All	P^1		
N	9	26	35	/	60	/
Age	65.55 ± 8.17	67.27 ± 10.39	67.05 ± 9.67	0.839	65.88 ± 8.24	0.526
Gender(M/F)	6/3	16/10	22/13	0.784	39/21	0.834
Underlying conditions						
Hypertension	6(66.67%)	15 (57.69%)	21 (60.00%)	0.712	44 (73.33%)	0.177
Diabetes	4(44.44%)	9(34.62%)	13(37.14%)	0.698	17(28.33%)	0.373
Coronary heart diseases	1(11.11%)	4(15.38%)	5(14.29%)	1.000	5(8.33%)	0.572
CKD	0(0.00%)	1(3.84%)	1(2.86%)	/	2(3.33%)	1.000
Liver diseases	2(22.22%)	1(3.84%)	3(8.57%)	0.156	2(3.33%)	0.354
Complications						
Double pneumonia	9(100%)	26(100%)	35(100%)	/	/	
hepatic injury	0(0.0%)	3(11.54%)	3(8.57%)	/	/	
Renal Dysfunction	1(11.11%)	1(3.45%)	2(5.71%)	0.454	/	
Intestinal diseases	1(16.67%)	3(11.54%)	4(11.43%)	1.000	/	
Treatment regimens						
Antiviral agent	8(88.89%)	26(100.00%)	34(97.14%)	/	/	
Antibiotic therapy	8(88.89%)	19(73.08%)	27(77.14%)	0.648	/	
Cellular immunotherapy	5(55.56%)	22(84.62%)	27(77.14%)	0.162	/	
Glucocorticoid therapy	1(11.11%)	10(38.46%)	11(31.43%)	0.217	/	
Chinese herb	7(77.78%)	22(84.62%)	29(82.86%)	0.635	/	

P^1 : Fibrosis group vs Non-fibrosis group;p^2:COVID-19 group vs Control group

Table 2
Symptoms and signs of 35 patients with COVID-19 Pneumonia at Day 1

Parameters	COVID-19 group (n = 35)			*P*
	Mild to moderate-fibrosis group	Severe-Fibrosis group	All(n = 35)	
Fever(°C)	1(16.67%),37.52 ± 1.32	5(17.24%),36.77 ± 0.77	6(26.08%),36.99 ± 0.98	0.240
Cough	5(83.33%)	27(91.30%)	32(91.43%)	0.442
Phlegm	5(83.33%)	19(65.52%)	24(68.57%)	0.640
Fatigue	4(66.67%)	27(93.10%)	31(88.57%)	0.128
Chest distress	4(66.67%)	23(85.19%)	27(77.14%)	0.602
Shortness of breath	3(50.00%)	25(86.21%)	28(80.00%)	0.079
mMRC scores	3.32 ± 0.40	3.24 ± 0.28	3.27 ± 0.32	0.539
SpO2(%)	93.27 ± 3.29	95.21 ± 3.20	94.60 ± 3.31	0.109
Oxygenation Index (OI)	303.27 ± 59.44	272.17 ± 59.69	283.97 ± 60.51	0.226

Baseline laboratory features comparison between groups

As shown in Table 3, most of the baseline laboratory characteristics did not differ between the COVID-19 and control groups as follows: routine blood indexes (white blood cell count [WBC]), inflammation indexes (C-reactive protein [CRP], IL-6, and erythrocyte sedimentation rate [ESR]), liver function (ALT, AST, ALP, and LDH), renal function (creatinine and urea), and myocardial enzymes (CK and CK-MB). The levels of PCT and ESR in the COVID-19 group (PCT: 0.08 ± 0.05 ng/L; ESR: 61.44 ± 39.61 mm/h) were higher than those in the control group (PCT: 0.04 ± 0.01 ng/L; ESR: 11.65 ± 6.38 mm/h), but did not differ between the mild-to-moderate fibrosis group (PCT: 0.07 ± 0.01 ng/L; ESR: 57.0 ± 4.24 mm/h) and severe fibrosis group (PCT: 0.08 ± 0.05 ng/L; ESR: 61.63 ± 42.34 mm/h). TGF-β and CCL18 levels were obviously increased in the patients, and higher in the severe fibrosis group (TGF-β: 206.47 ± 165.23 pg/mL; CCL18: 366.64 ± 161.06 ng/mL) than in the mild-to-moderate fibrosis group (TGF-β: 88.36 ± 97.45 pg/mL, *P* = 0.024; CCL18: 241.84 ± 125.37 ng/mL, *P* = 0.038). Similarly, levels of P□P, HA, LN, and C□ were higher in the patients with COVID-19 than in the control group (P□P: 57.27 ± 57.53 vs 17.15 ± 2.48 ng/mL, *P* = 0.000; HA: 121.05 ± 80.71 vs 57.72 ± 17.23 ng/mL, *P* = 0.003; LN: 59.77 ± 48.65 vs 24.00 ± 7.49 ng/mL, *P* = 0.000; C□: 24.49 ± 13.86 vs 15.38 ± 1.15 ng/mL, *P* = 0.000), but did not differ between the mild-to-moderate fibrosis group and the severe fibrosis group.

Table 3
Laboratory tests of 35 patients with COVID-19 Pneumonia at Day 1 and Control individuals.

Parameters	COVID-19 group (n = 35)				Control group(n = 60)	P^2
	mild to moderate-fibrosis group	Severe-Fibrosis group	All	P^1		
Blood routine index						
WBC (109 /L)	5.79 ± 1.38	5.91 ± 1.21	5.87 ± 1.24	0.815	5.63 ± 1.03	0.444
N (109 /L)	3.69 ± 1.15	3.40 ± 1.01	3.49 ± 1.04	0.191	3.13 ± 0.78	0.169
L (109 /L)	1.42 ± 0.39	1.73 ± 0.62	1.62 ± 0.56	0.508	1.65 ± 0.36	0.829
Inflammation index						
CRP (mg/L), median (IQR)	1.61(0.50,6.10)	1.34(0.50,3.25)	1.34(0.50,3.70)	0.886	1.97(0.80,4.72)	0.215
PCT (ng/L)	0.10 ± 0.09	0.07 ± 0.04	0.08 ± 0.05	0.537	0.04 ± 0.01	0.001
ESR (mm/h)	57.0 ± 4.24	61.63 ± 42.34	61.44 ± 39.61	0.886	11.65 ± 6.38	0.005
IL-6, median (IQR)	2.12(0.19,8.80)	1.86(0.05,7.92)	1.86(0.01,7.82)	0.784	4.21(1.69,3.70)	0.152
Liver function						
ALT(U/L)	35.50 ± 14.08	32.14 ± 23.18	32.20 ± 20.40	0.749	31.04 ± 12.87	0.689
AST(U/L)	30.56 ± 9.91	25.10 ± 10.87	26.82 ± 10.62	0.311	25.88 ± 9.48	0.758
LDH(U/L)	240.90 ± 37.67	204.12 ± 33.26	213.65 ± 37.49	0.123	212.48 ± 51.83	0.926
ALP(U/L)	80.20 ± 16.42	74.92 ± 22.60	75.29 ± 21.00	0.578	75.16 ± 25.50	0.863
Renal function						
sCr(μmol/L)	50.07 ± 15.56	50.98 ± 12.09	50.74 ± 12.76	0.876	56.70 ± 15.53	0.122
UREA (mmol/L)	4.89 ± 1.35	5.14 ± 1.57	5.07 ± 1.50	0.719	5.04 ± 1.41	0.939
Myocardial enzymes						
CK(U/L)	36.28 ± 8.04	60.37 ± 60.18	51.34 ± 48.37	0.352	55.48 ± 17.68	0.746
CK-MB (U/L)	5.44 ± 3.21	7.72 ± 4.27	6.32 ± 3.94	0.225	6.84 ± 4.14	0.898

WBC, white blood cell; N, neutrophils; L, lymphocytes; CRP, c-reactive protein; PCT, procalcitonin; ESR, erythrocyte sedimentation rate;IL-6, interleukin- 6; ALT, alanine aminotransferase; AST, aspartate aminotransferases; LDH, lactate dehydrogenase; ALP, alkaline phosphatase sCr, serum creatinine; CK, creatine kinase; CK-MB ,creatine kinase isoenzyme MB; PIIIP, precollagen III peptide; HA, hyaluronidase; LN, laminin; CIV ,collagen type IV; TGF-β, transforming growth factor-β;CCL18, CC-chemokine ligand 18.

Parameters	COVID-19 group (n = 35)				Control group(n = 60)	P^2
	mild to moderate-fibrosis group	Severe-Fibrosis group	All	P^1		
Fibrosis index						
P□P(ng/mL)	51.99 ± 28.26	59.38 ± 66.12	57.27 ± 57.53	0.737	17.15 ± 2.48	0.000
HA(ng/mL)	92.78 ± 23.51	131.22 ± 91.47	121.05 ± 80.71	0.063	57.72 ± 17.23	0.000
LN(ng/mL)	81.17 ± 75.34	52.92 ± 36.04	59.77 ± 48.65	0.156	24.00 ± 7.49	0.000
C□(ng/mL)	29.32 ± 20.97	21.46 ± 10.27	24.49 ± 13.86	0.171	15.38 ± 1.15	0.003
TGF-β(pg/mL)	88.36 ± 97.45	206.47 ± 165.23	169.81 ± 155.97	0.024	57.23 ± 24.92	0.000
CCL18(ng/mL)	241.84 ± 125.37	366.64 ± 161.06	328.82 ± 160.14	0.038	84.47 ± 60.67	0.000

WBC, white blood cell; N, neutrophils; L, lymphocytes; CRP, c-reactive protein; PCT, procalcitonin; ESR, erythrocyte sedimentation rate;IL-6, interleukin- 6; ALT, alanine aminotransferase; AST, aspartate aminotransferases; LDH, lactate dehydrogenase; ALP, alkaline phosphatase sCr, serum creatinine; CK, creatine kinase; CK-MB ,creatine kinase isoenzyme MB; PIIIP, precollagen III peptide; HA, hyaluronidase; LN, laminin; CIV ,collagen type IV; TGF-β, transforming growth factor-β;CCL18, CC-chemokine ligand 18.

Characteristics of fibrosis in follow-up patients with COVID-19 pneumonia

All fibrosis parameters and thin-section chest CT scans were repeated after 1 week, 2 weeks, 1 month, 2 months, and 3 months. The lung function indexes included SpO2, oxygenation index (OI), and mMRC scores were also followed up.

Ground-glass opacities and subsegmental areas of consolidation were found on initial chest CT imaging, which were the typical imaging features of COVID-19 pneumonia. As shown in Fig. 2, the radiologic changes included ground-glass opacities, fibrotic stripes, architectural distortion, isolated areas of pleural thickening, reticular patterns, and traction bronchiectasis. The changes were further assessed by HRCT scores. After following up for 90 days, we found that the scores gradually decreased during the follow-up period, from 22.48 ± 16.13 to 10.33 ± 11.11 on Day 90 (P□0.001), showing the improvement of pulmonary fibrosis.

The other fibrosis parameters were also followed up. As shown in Table 4, the SpO_2 and OI, without supplementary oxygen, rose from 95.11 ± 3.25% (Day 1) to 97.09 ± 1.95% (Day 30) and from 288.97 ± 65.05 (Day 30) to 386.89 ± 48.84 (Day 30), respectively (both $P < 0.001$). Meanwhile, the mMRC scores decreased from 3.27 ± 0.32 (Day 1) to 1.48 ± 0.33 (Day 90), which indicated that the pulmonary alveoli regained gas-exchange function and lung function recovered gradually. Moreover, with the improvement of pulmonary fibrosis, the level of TGF-β decreased to normal levels in 90 days ($P < 0.001$), and the CCL18 level decreased from 364.27 ± 167.70 ng/mL to 185.57 ± 105.29 ng/mL ($P < 0.001$), but was still higher than normal levels. The other fibrosis indexes, including HA, LN, and C□, reached normal reference values, namely, 56.19 ± 17.71 U/L,

29.73 ± 15.25 U/L, and 16.84 ± 2.81 U/L, respectively. However, P□P showed no differences during the 90 days of follow-up ($P = 0.312$) and remained at higher than normal levels ($P < 0.001$).

Table 4
clinical characteristics of 35 patients with COVID-19 Pneumonia during further follow-up for 90 days.

clinical characteristics	Follow-up time						*P*
	Day1	Day7	Day14	Day30	Day60	Day90	
SpO2(%)	94.60 ± 3.31	95.66 ± 2.98	96.66 ± 2.15	97.06 ± 2.01	/	/	0.000
Oxygenation Index(OI)	284.50 ± 60.51	317.93 ± 60.70	342.29 ± 56.54	386.048.84	/	/	0.000
mMRC scores	3.27 ± 0.32	3.24 ± 0.35	3.20 ± 0.19	2.57 ± 0.33	2.10 ± 0.34	1.48 ± 0.33	0.001
HRCT scores	22.48 ± 16.13	20.85 ± 16.02	20.15 ± 14.94	11.58 ± 9.95	11.63 ± 9.46	10.33 ± 11.11	0.000
TGF-β(pg/mL)	169.81 ± 155.97	112.52 ± 113.08	63.83 ± 44.25	50.66 ± 33.98	33.88 ± 22.91	19.95 ± 12.74	0.001
CCL18(ng/mL)	364.27 ± 167.70	301.77 ± 160.87	323.16 ± 140.89	221.77 ± 136.54	194.69 ± 139.84	185.57 ± 105.29	0.000
P□P	53.72 ± 36.20	48.29 ± 37.51	48.74 ± 24.37	45.88 ± 25.24	48.84 ± 34.79	45.75 ± 26.23	0.312
HA	121.05 ± 80.71	101.78 ± 50.41	96.13 ± 35.29	81.86 ± 37.14	75.09 ± 23.91	57.48 ± 11.88	0.048
LN	59.77 ± 48.65	42.05 ± 25.17	39.73 ± 21.31	35.21 ± 16.29	31.62 ± 10.90	29.73 ± 15.25	0.007
C□	23.49 ± 13.86	23.05 ± 9.00	23.43 ± 9.17	13.61 ± 4.05	16.20 ± 3.24	16.84 ± 2.81	0.005

As shown in Fig. 2, the patients in the fibrosis groups were further assessed and analyzed. We found that the scores for mMRC and HRCT decreased gradually in both groups. The levels of TGF-β and CCL18 decreased gradually in the severe fibrosis group, but showed no obvious changes in the mild-to-moderate fibrosis group.

Prediction values of TGF-β and CCL18 in pulmonary fibrosis patients

In addition, based on reduction of the HRCT score to 10, the probability of 90-day prognosis between two groups divided according to the serum baseline levels of TGF-β and CCL18 was compared using the Kaplan-Meier method. Cut-off levels of TGF-β and CCL18 of 82 pg/mL and 286 ng/mL, respectively, according to their ROC curves, were used to discriminate severe fibrosis patients from mild-to-moderate fibrosis patients [see Additional file 1] .The probability of 90-day prognosis differed between the two groups, and higher levels of TGF-β and CCL18 indicated a longer recovery time. With log-rank Mantel–Cox analysis, only TGF-β (hazard

ratio [HR] 2.799, *P* = 0.024) and CCL18 (HR 2.894, *P* = 0.041) were shown to be independent predictors for pulmonary fibrosis in patients with COVID-19.

Discussion

At present, most patients with COVID-19 have been discharged after appropriate medical treatments, but there remains concern that lung injury, including pulmonary fibrosis, might lead to long-term impairment following infection. In our study, we paid attention to the follow-up of these recovered patients with COVID-19. Clinical features between patients with mild-to-moderate or severe fibrosis were compared, and we attempted to identify monitors and predictors for the development of pulmonary fibrosis by combining follow-up clinical data with disease progression during 90 days .

In this study, 35 recovered patients with COVID-19 were followed up, of which 76.29% (26/35) displayed severe fibrosis on CT images. This finding further suggested that fibrosis might be a familiar comorbidity in COVID-19. Laboratory testing and clinical evaluation were performed to identify the progression of the disease. Although many routine studies have shown that routine blood indexes (leuko- and lymphopenia), liver enzymes (mildly elevated LDH and ALP), and inflammatory indicators (increased levels of CRP and IL-6) have good diagnostic values and are associated with pneumonia and extensive lung damage[4, 16, 17], most of them show no obvious changes due to mild inflammatory reactions during recovery. This is not consistent with an associated study, which showed that compared with control individuals, levels of PCT, ESR, and all fibrosis indicators (TGF-β, CCL18, PIIIP, HA, LN, and CIV) were increased in patients with COVID-19. PIIIP, HA, LN, and CIV have been applied widely as serum markers of hepatic fibrosis in clinical practice[18, 19]. Recent reports have indicated that levels of these indicators are also increased in patients with idiopathic pulmonary fibrosis (IPF), which reflected its prognosis and severity[20]. Pulmonary fibrosis is the end-stage pathological changes of many lung diseases characterized by the proliferation of fibroblasts, excessive deposition of extracellular matrix (ECM) and unsuccessful reconstruction of the destructed alveolar epithelium, as well as inflammatory damage and normal lung structure destruction. PIIIP, HA, LN, and CIV are associated with the ECM, suggesting their potential as diagnostic biomarkers for pulmonary fibrosis[21]. In our study, although there were no obvious differences between the mild-to-moderate fibrosis group and the severe fibrosis group because of the small sample size at the initial observation point, HA, LN, and CIV gradually decreased with the improvement of fibrosis during the 90 days' follow-up. These results further indicate their potential as monitoring biomarkers for pulmonary fibrosis in patients with COVID-19, yet larger sample's cohort studies, as well as further prospective studies, are needed to prove this point.

In addition to the above mentioned four fibrosis indicators, numerous reports have indicated that TGF-β (anti-inflammatory cytokine) and CCL18 (chemokine) are closely related to extensive lung injury and pneumonia fibrosis[22, 23]. In our study, baseline serum levels of the two markers were obviously increased in patients with COVID-19. During the follow-up period, elevated levels of serum TGF-β and CCL18 were associated with pulmonary dysfunction and poorer prognosis of pneumonia fibrosis. COVID-19 is ascribed to "cytokine storm" characterized by largely uncontrolled inflammatory responses [24]. Nevertheless, few published reports have displayed circulatory concentration of TGF-β and CCL18 in patients with COVID-19, and most focus on pro-and anti-inflammatory cytokines such as IL-6, TNF-α, IL-1β, and so on. In this respect, our findings supplement the doctrine of a cytokine storm in COVID-19. In addition, our study also illustrated that circulatory levels of TGF-β

and CCL18 were capable of differentiating severe fibrosis patients from mild subjects with COVID-19, suggesting their potential as evaluation and monitoring biomarkers.

Generally, it is considered that TGF-β is a central medium of the repair phase of tissue damage, adjusting immunity and inflammation response and promoting scar formation. Series of studies on acute pulmonary injury thoroughly evaluated the role of TGF-β during the late phases of tissue repair and demonstrate that it is associated with the development of pulmonary fibrosis[25]. It plays a critical role in epithelial–mesenchymal interactions during alveolarization and lung branching morphogenesis, and involve in the regulation of multiple cellular processes, such as the differentiation of alveolar epithelial cell, the proliferation of fibroblasts, excessive deposition of ECM, and growth inhibition of epithelial cells[26]. In patients with COVID-19, large numbers of neutrophils are infiltrated into the lung tissues by the virus and release stored TGF-β resulting in apoptosis of numerous cells, such as pneumocytes, bronchial epithelial cells, T-lymphocytes, and even neutrophils themselves [27]. Consequently, it induces more macrophages migration and infiltration into the lungs, with the resultant production and secretion of large amounts of TGF-β, which leads to tissue remodeling of lung injury in COVID-19[28, 29]. These may be the main possible sources of massive increases in TGF-β.

CCL18 as a CC-chemokine is derived from alveolar macrophages and acts as a chemoattractant. Some studies reported that CCL18 could promote collagen production and induce chemotaxis of lung fibroblasts in vitro[30]. In IPF, serum CCL18 is increased and negatively correlated to pulmonary function[23, 31], which is consistent with our results in COVID-19. Another prospective study also demonstrated that CCL18 was an independent factor related to the mortality of IPF patients (HR 1.98, 95% CI 2.49–25.51, $P < 0.05$)[32]. Similarly, elevated CCL18 levels are associated with poorer prognosis of pneumonia fibrosis[23, 33]. Therefore, we speculated that the uncontrolled and sudden increases of TGF-β and CCL18 in COVID-19 (possibly accompanied with other inflammatory cytokines) may participate in progression to fibrosis. Therefore, TGF-β and CCL18 blockade may be beneficial for COVID-19 patients.

Certainly, there are several limitations in the present study. Initially, the sample size of 35 patients was relatively small, which could have lead to some potentially useful biomarkers or clinical characteristics being missed. Furthermore, only severe COVID-19 patients were included in this study, and fibrosis indexes in asymptomatic or mild patients were not further followed up. This might have introduced a selection bias for some data. Finally, control composition only included the subjects without pneumonia. Patients with other lung diseases, especially IPF, were not included. Thus, the diagnostic and predictive utility of these indicators mentioned in this study were not fully evaluated.

Conclusion

The pulmonary fibrosis of most patients with COVID-19 gradually improved over time. Our results showed that, in addition to fibrosis indicators (HA, LN, and CIV), both TGF-β and CCL18 could differentiate pulmonary fibrosis patients with COVID-19 from control subjects and gradually declined with the improvement of fibrosis during 90 days of follow-up. We also found that elevated TGF-β and CCL18 levels were associated with poorer prognosis of pneumonia fibrosis. These results indicate that both these parameters may be promising biomarkers for predicting and monitoring the pulmonary fibrosis in patients with COVID-19.

Abbreviations

TGF-β, transforming growth factor beta;

CCL18,chemokine ligand 18

P□P, type III procollagen peptide

HA, hyaluronic acid

LN, laminin

C□,type IV collagen

HRCT, high-resolution computed tomography

mMRC, modified Medical Research Council

SARS, severe acute respiratory syndrome

ARDS, acute respiratory distress syndrome

OI, oxygenation index

IPF, idiopathic pulmonary fibrosis

ECM, extracellular matrix

Declarations

Acknowledgments

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Authors' contributions

MZ, QL, LF and ZL participated in the study design. HZ, LL, LH and XW were involved in the conduct of the study and data collection. MZ, LZ, EJ and YS made contributions to data analysis and results interpretation.NZ, LW, JY and LL participated in the detecting of ELISA kits. MZ, LZ, LF and ZL wrote and modified the manuscript and prepared tables and figures. All agreed to be accountable for all aspects of the work.

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Availability of data and materials

Data are however available from the authors upon reasonable request.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Tong Ji University (DFSC-2019(CR)-03), and informed consent was obtained from all the subjects prior to the study.

Consent for publication

Neither the entire paper nor any part of paper has been published or has been accepted elsewhere.All authors have approved the final manuscript and agreed to publication in the journal.

Competing interests statement

There are no competing interests.

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Figures

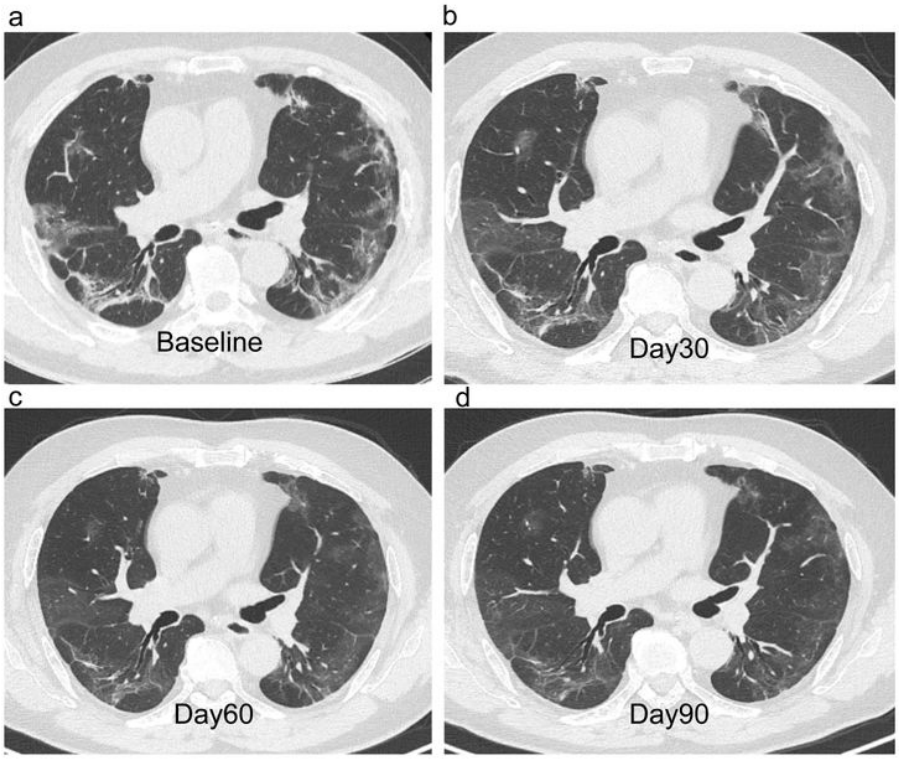

Figure 1

Typical CT imaging findings of 53-year-old woman with confirmed COVID-19 pneumonia. A. First thin-section chest CT scan in hospital on March 9, 2020 (20 days after symptoms onset). CT imaging showed ground-glass opacity fibrotic strips coarse reticular pattern and interstitial thickening in bilateral lobes. Traction

bronchiectasis and adjacent pleural retraction were obvious. B. Thirty days later, extent of lesions was decreased. Ground-glass opacity was decreased. Fibrotic strips and traction bronchiectasis still existed. C. Sixty days later, CT imaging showed ground-glass opacity was decreased and fibrotic strips were not improved. D. Ninety days later, residual ground-glass opacity was further decreased and fibrotic strips were still observed.

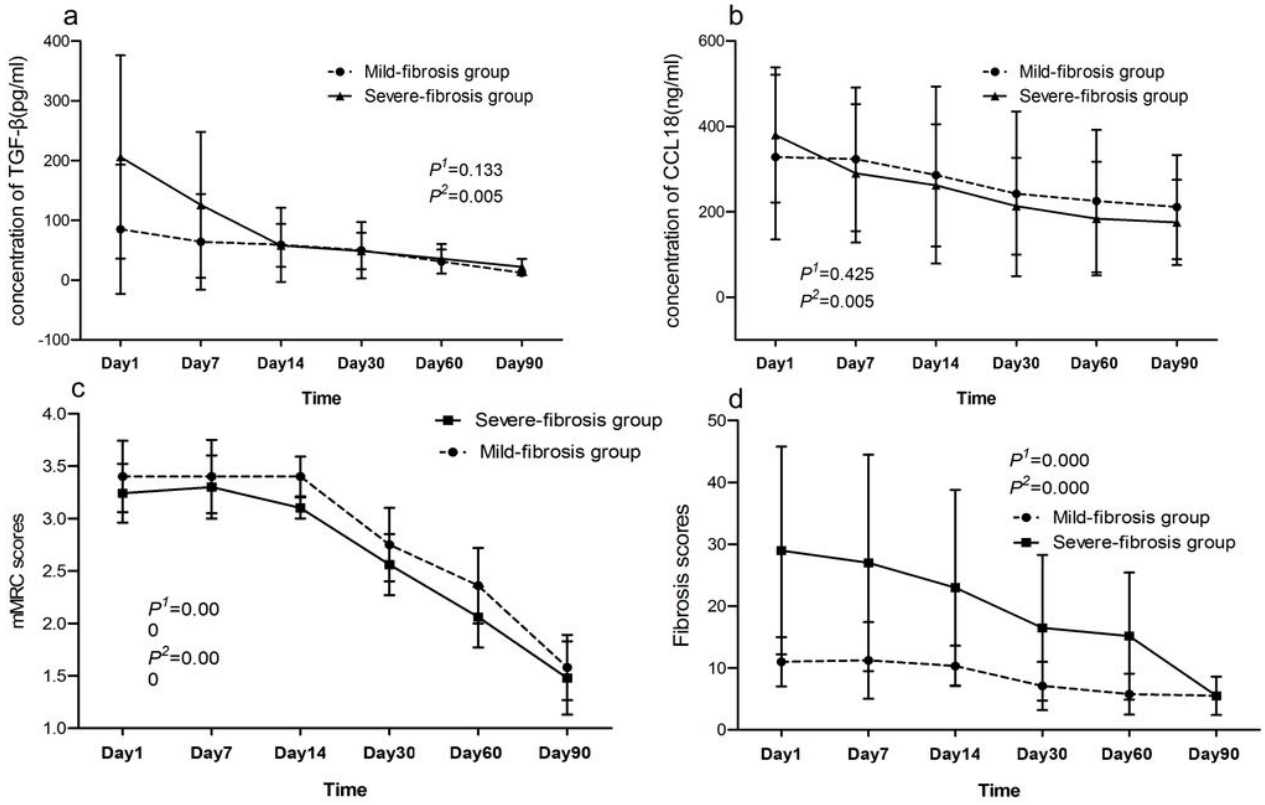

Figure 2

TGFβ, CCL18, mMRC scores and HRCT scores of 35 patients with COVID-19 pneumonia during further follow-up for 90 days. The patients from the different fibrosis group were further assessed and analyzed respectively. The levels of TGF-βand CCL18 were also decreased gradually in the severe-fibrosis group, but showed no obvious changes in mild to moderate -fibrosis group (A and B). The scores of mMRC and HRCT were decreased gradually in both groups (C and D).

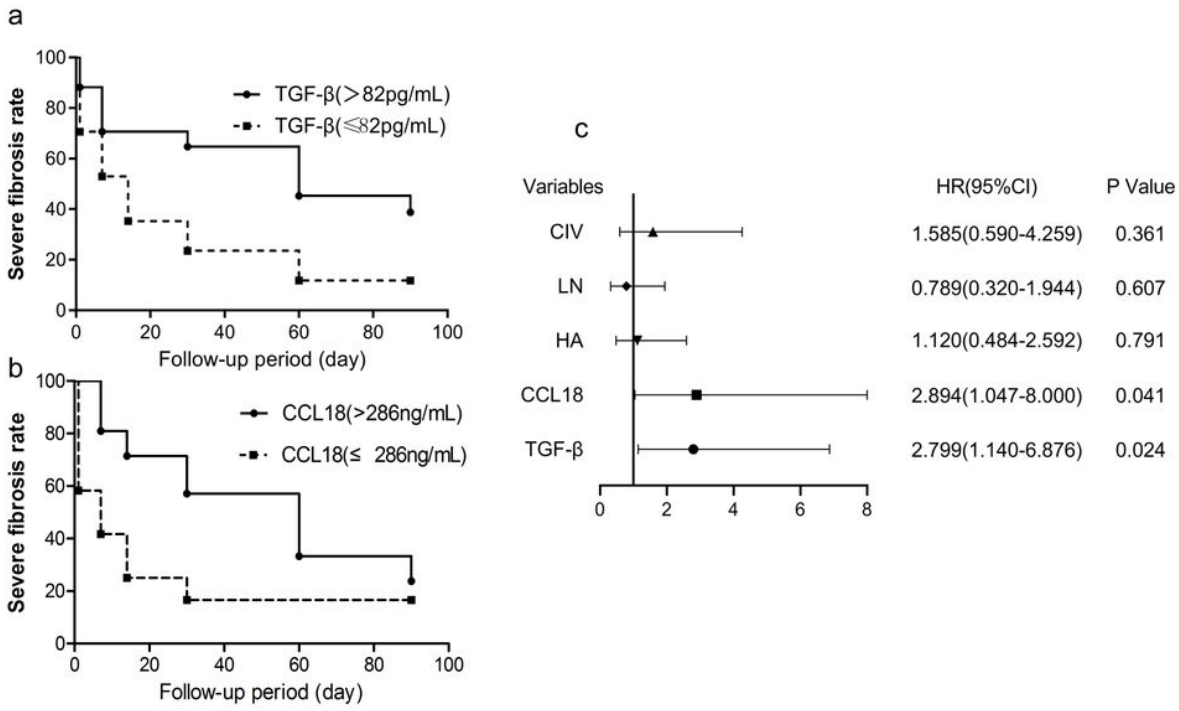

Figure 3

Kaplan-Meier analysis to evaluate the probability of 90-day prognosis among the two groups which were divided according to the serum levels of TGF-β and CCL18.The cut-off levels of TGF-β and CCL18 were 82 Pg/mL and 286 ng/mL, respectively. A. the prognostic curve of the fibrosis patients using TGF-β level above or below the cut-off level; B. the prognostic curve of the fibrosis patients using CCL18 level above or below the cut-off level; C. the log rank Mental -Cox analysis of the fibrosis indexes.

Supplementary Files

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- FigS1.jpg
- FigS2.jpg