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Dynamics of cytokines and lymphocyte subsets associated with the poor prognosis of severe COVID-19

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Abstract. – OBJECTIVE: We aimed to study the dynamics of cytokines and lymphocyte subsets and their correlation with the prognosis of patients with severe COVID-19.

PATIENTS AND METHODS: The lymphocyte subsets and cytokines of 31 patients with severe COVID-19 (7 deaths and 24 survivals) were longitudinally analyzed.

RESULTS: The mean age of enrolled patients was 64 years, 24 (77.4%) patients were men, and 23 (74.2%) patients had comorbidities. Compared with survival group, the death group showed significant and sustained increases in the levels of IL-6, IL-8, and IL-10 from baseline to 28 days after admission (all $p<0.05$). No significant differences were observed in the levels of TNF-α, IL-1β, IL-2, IL-4, IL-5, IL-12P70, IL-17, IFN-α, and IFN-γ between the death group and survival group during the follow-up (all $p>0.05$). The absolute counts of CD3+ T cells, CD4+ T cells, CD8+ T cells, and CD45+ T cells were lower in both survival group and death group patients from hospital admission to 3 days after admission, and gradually recovered in 4 to 35 days in the survival group, but continually stayed at low levels in the death group during the follow-up.

CONCLUSIONS: The kinetic changes of cytokines and lymphocyte subsets are related with the prognosis of patients with severe COVID-19.

Key Words:
Severe acute respiratory syndrome coronavirus 2, 2019 Novel coronavirus disease, Lymphocyte subsets, Cytokine profiles, Disease prognosis.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an RNA virus which can cause 2019 novel coronavirus disease (COVID-19)[1]. The outbreak of COVID-19 has caused a global pandemic. As of July 28th, 2020, there have been 16341,920 confirmed cases and 650,805 deaths globally[2]. The disease spectrum of COVID-19 ranges from mild self-limiting disease to severe pneumonia, which might cause acute respiratory distress syndrome (ARDS), and death[3]. The severity of COVID-19 depends on the efficiency of the immune system which, if it is weak, cannot stem the SARS-CoV-2 infection and its symptoms.

Previous studies[4,5] have showed that lymphopenia and inflammatory cytokine storm are typical laboratory abnormalities observed in patients with COVID-19, and are associated with the disease severity. Chen et al[4] enrolled 21 patients with COVID-19 and found that compared with moderate cases, severe cases had markedly higher levels of IL-2R, IL-6, IL-10, and TNF-α. Absolute numbers of T lymphocytes, CD4+ T cells, and CD8+ T cells decreased in nearly all patients with COVID-19, and were markedly lower in severe cases than moderate cases[4]. Liu et al[5] found significant decreases in the counts of T cells, especially CD8+ T cells, as well as increases in IL-6, IL-10, IL-2 and IFN-γ levels in the severe cases compared to those in the mild cases. The authors concluded that the degree of lymphopenia and cytokine storm is higher in severe patients than in mild cases and is associated with the disease severity of COVID-19.

With the publication of more and more clinical data, a large number of data suggest that there are mild or severe cytokine storms and lymphopenia in patients with severe COVID-19. However, it has remained largely unclear in the dynamic changes of cytokines and lymphocyte subsets, and their

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correlation with the prognosis of patients with severe COVID-19. In this study, we longitudinally studied the dynamics of cytokine profiles and lymphocyte subsets and explored whether the dynamic changes is an important cause of death in patients with severe COVID-19.

Patients and Methods

Patients

A total of 31 patients with severe COVID-19 admitted to Shanghai Public Health Clinical Center, Shanghai, China, from January 20th, 2020 to June 23rd, 2020, were enrolled into this study. Shanghai Public Health Clinical Center is a tertiary teaching hospital in China and is designated by the Chinese government for the treatment of patients with COVID-19. Patients with COVID-19 were diagnosed according to the positive results of SARS-COV-2 nucleotides tests in the nasopharyngeal or throat swab specimens[6]. Severe cases were defined as at least one of the followings[7]: (1) Respiratory distress, respiratory rates ≥ 30/min; (2) Pulse oxygen saturation ≤ 93% in a resting state; (3) Oxygenation index (PaO_2/FiO_2) ≤ 300 mmHg; (4) Require mechanical ventilation; (5) shock; (6) Combined with other organ failures and needed treatment in the intensive care unit (ICU).

Laboratory Examination

The SARS-CoV-2 nucleic acids were detected using automatic magnetic extraction device and accompanying kit (Bio-Germ Medical Technology Co., Ltd, Shanghai, China) and screened with a semi-quantitative real-time polymerase-chain-reaction (RT-PCR) kits (Bio-Germ Medical Technology Co., Ltd, Shanghai, China) with amplification targeting the ORF1a/b and N gene. The lymphocyte test kits (Becton Dickinson and Company, San Diego, CA, USA) were used for lymphocyte subset analysis. Twelve plasma cytokines were detected using the human cytokine kit II (Raisecare Ltd, Qingdao, China). All tests were performed according to the product manual.

Data Collection

The demographic characteristics, epidemiological characteristics, clinical manifestations, laboratory tests, and clinical outcomes were extracted from the electronic medical records. The data of cytokines and lymphocyte subsets were obtained from hospital admission to 35 days after admission.

Statistical Analysis

The normality test was performed for continuous variables using the Kolmogorov-Smirnov test. Normal distribution variables were expressed as mean ± standard deviation and compared using the *t*-test. Non-normal distribution continuous variables were presented as medians [interquartile ranges (IQR)] and compared with the Mann-Whitney test. Categorical variables were showed as numbers (percentage) and compared by the chi-square test. All significance tests were two-tailed, and $p < 0.05$ was considered statistically significant. All statistical analyses were done using the SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Demographics and Clinical Characteristics of Patients with Severe COVID-19

Demographics and clinical characteristics of patients with severe COVID-19 are shown in Table I. The age of patients was 64±14 years, 24 (77.4%) were men, and 23 (74.2%) had comorbidities. The common comorbidities included hypertension (16 [51.6%]), diabetes (8 [25.8%]), chronic pulmonary disease (3 [9.7%]), cardiovascular disease (7 [22.6%]), cerebrovascular disease (2 [6.5%]), chronic kidney disease (2 [6.5%]), and malignancy (1 [3.2%]). The common symptoms were fever (26 [83.9%]), cough (15 [48.4%]), dyspnea (9 [29.0%]), and fatigue (6 [19.4%]).

All patients received antiviral agents including Lopinavir/Ritonavir, Oseltamivir, Emtricitabine/Tenofovir, Interferon-alpha, Hydroxychloroquine, Arbidol, and Chinese medicine. 26 patients (83.9%) received corticosteroids therapy, 27 patients (87.1%) received intravenous immunoglobulin therapy, and 26 patients (83.9%) received thymosin-α1 therapy. There was no statistic difference in the demographics and clinical characteristics between the survival group and death group, besides cerebrovascular disease (28.6% *vs.* 0, p=0.007) and chronic kidney disease (28.6% *vs.* 0, p=0.007) (Table I).

Laboratory findings of Patients with Severe COVID-19

Laboratory findings of patients with severe COVID-19 are shown in Table II. The median levels of white blood count (WBC), lymphocyte count, aspartate aminotransferase, total bilirubin, blood urea nitrogen, creatinine, creatine kinase, D-dimer, and procalcitonin were 4.9 (3.5-

Table I. Demographics and clinical characteristics of patients with severe COVID-19.

	All (n=31)	Survival (n=24)	Death (n=7)	*p*-value
Characteristics				
Age (years)	64±14	62±12	70±21	0.178
Male, n (%)	24 (77.4%)	19 (79.2%)	5 (71.4%)	0.667
Any comorbidity, n (%)	23 (74.2%)	18 (75%)	5 (71.4%)	0.849
Hypertension	16 (51.6%)	13 (54.2%)	3 (42.9%)	0.598
Diabetes mellitus	8 (25.8%)	8 (33.3%)	0	0.076
Chronic pulmonary disease	3 (9.7%)	2 (8.3%)	1 (14.3%)	0.639
Cardiovascular disease	7 (22.6%)	4 (16.7%)	3 (42.9%)	**0.145**
Cerebrovascular disease	2 (6.5%)	0	2 (28.6%)	**0.007**
Chronic kidney disease	2 (6.5%)	0	2 (28.6%)	0.007
Malignancy	1 (3.2%)	0	1 (14.3%)	0.060
Signs and symptoms, n (%)				
Fever	26 (83.9%)	19 (79.2%)	7 (100%)	0.187
Cough	15 (48.4%)	13 (54.2%)	2 (28.6%)	0.233
Dyspnea	9 (29.0%)	7 (29.2)	2 (28.6%)	0.976
Fatigue	6 (19.4%)	4 (16.7%)	2 (28.6%)	0.483
Treatments, n (%)				
Antiviral therapy	31 (100%)	24 (100%)	7 (100%)	
Corticosteroids	26 (83.9%)	20 (83.3%)	6 (86.7%)	0.880
IVIG	27 (87.1%)	20 (83.3%)	7 (100%)	0.247
Thymosin-α1	26 (83.9%)	21 (87.5%)	5 (71.4%)	0.309

IVIG, intravenous immunoglobulin; The *p*-values indicate differences between survival group and death group. $p<0.05$ was considered statistically significant.

7.9)×10^9/L, 0.7 (0.5-0.9)×10^9/L, 44 (24-52) U/L, 10.3 (8.6-14.2) μmol/L, 5.2 (4.1-9.3) mmol/L, 80 (56-98) μmol/L, 209 (113-365) U/L, 1.2 (0.6-1.9) ng/mL, and 0.12 (0.05-0.53) ng/mL, respectively. The mean levels of alanine aminotransferase, lactate dehydrogenase, and C-reactive protein were 32±18 U/L, 405±138 U/L, and 55±45 mg/L, respectively. There was no statistic difference in the laboratory findings between the survival group and death group (all $p > 0.05$).

Table II. Laboratory findings of patients with severe COVID-19.

	All patients (n=31)	Survival group (n=24)	Death group (n=7)	*p*-value
WBC (10^9/L)	4.9 (3.5-7.9)	5.9 (3.6-9.6)	4.2 (3.2-6.1)	0.375
Lymphocyte (10^9/L)	0.7 (0.5-0.9)	0.8 (0.4-1.0)	0.6 (0.5-0.7)	0.229
ALT (U/L)	32±18	33±20	30±14	0.713
AST (U/L)	44 (24-52)	33 (24-52)	48 (28-59)	0.163
TBIL (μmol/L)	10.3 (8.6-14.2)	10.0 (8.2-13.6)	13.8 (10.3-22.1)	0.082
BUN (μmol/L)	5.2 (4.1-9.3)	5.2 (3.6-8.5)	5.2 (4.2-11.8)	0.317
Creatinine (μmol/L)	80 (56-98)	77 (54-89)	118 (57-176)	0.194
CK (U/L)	209 (113-365)	165 (111-276)	350 (225-436)	0.126
LDH (U/L)	405±138	382±129	484±150	0.086
D-dimer (ng/mL)	1.2 (0.6-1.9)	1.0 (0.5-1.8)	1.5 (0.7-2.0)	0.346
PCT (ng/mL)	0.12 (0.05-0.53)	0.10 (0.04-0.41)	0.24 (0.06-1.21)	0.078
CRP (mg/L)	55±45	35 (14-77)	51±47	0.445

WBC, white blood count; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; BUN, blood urea nitrogen; CK, creatine kinase; LDH, lactate dehydrogenase; PCT, procalcitonin; CRP, C-reactive protein; the *p*-values indicate differences between survival group and death group. $p < 0.05$ was considered statistically significant.

Dynamic Changes of Cytokines in Patients with Severe COVID-19

In this study, we combined the longitudinal cytokines data of the death group and survival group and plotted their fluctuation patterns against the time point after admission (Figure 1). Compared with the survival group, the death group showed significant and sustained increases in the serum levels of IL-6, IL-8, and IL-10 from hospital admission to 28 days after admission (all $p < 0.05$) (Figure 1 and Table III) and recovered to levels that were comparable to those of the survival group at 29 to 35 days after admission (all $p > 0.05$) (Figure 1). No significant differences were observed in the levels of TNF-α, IL-1β, IL-2, IL-4, IL-5, IL-12P70, IL-17, IFN-α, and IFN-γ between the death group and survival group during the follow-up (all $p > 0.05$) (Figure 1).

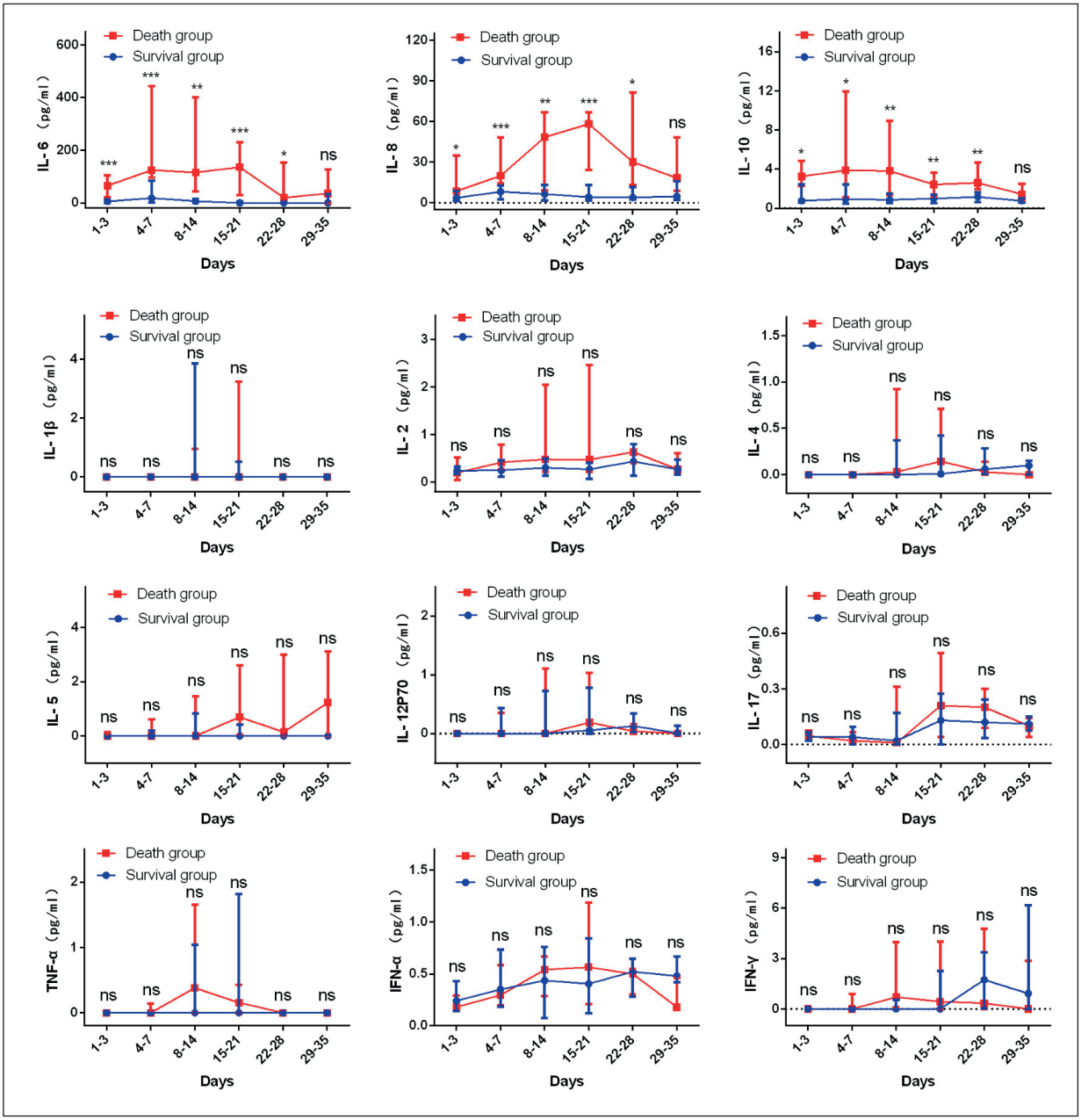

Figure 1. Kinetic analysis of cytokines levels in patients with severe COVID-19. The cytokines levels in the survival group (blue line) and death group (red line) patients were analyzed at different time points after hospital admission. The levels of cytokines are shown using median and interquartile range.

Table III. Comparison in the levels of cytokines between death group and survival group.

		Death group (n=7)	Survival group (n=24)	*p*-value
IL-6 (pg/ml)	day 1-3	64.98 (22.27-104.71)	5.61 (0-12.31)	**<0.001**
	day 4-7	124.37 (95.61-443.95)	17.45 (0.37-84.28)	**<0.001**
	day 8-14	115.53 (42.85-401.54)	6.52 (0-16.18)	**0.002**
	day 15-21	135.76 (29.01-230.55)	0 (0-5.66)	**<0.001**
	day 22-28	19.57 (0.50-153.70)	0 (0-3.86)	**0.040**
	day 29-35	35.34 (0-127.66)	0 (0-33.81)	0.258
IL-8 (pg/ml)	day 1-3	8.66 (7.54-34.93)	3.70 (1.39-8.9)	**0.015**
	day 4-7	19.93 (14.50-48.29)	8.28 (2.80-12.82)	**<0.001**
	day 8-14	48.49 (9.25-66.81)	6.59 (1.75-13.07)	**0.004**
	day 15-21	58.24 (24.18-67.01)	4.21 (1.92-13.27)	**<0.001**
	day 22-28	30.15 (13.03-81.37)	4.09 (2.37-11.86)	**0.010**
	day 29-35	18.35 (8.68-48.49)	4.85 (2.26-15.94)	0.073
IL-10 (pg/ml)	day 1-3	3.00 (0.32-4.84)	0.78 (0.59-2.43)	**0.013**
	day 4-7	3.89 (1.13-11.94)	0.95 (0.51-2.44)	**0.024**
	day 8-14	3.82 (1.35-8.94)	0.86 (0.54-1.51)	**0.001**
	day 15-21	2.44 (1.26-3.66)	1.00 (0.52-1.44)	**0.009**
	day 22-28	2.16 (1.04-4.67)	1.17 (0.63-1.60)	**0.009**
	day 29-35	1.49 (0.88-8.11)	0.76 (0.60-1.04)	0.073

The *p*-values indicate differences between death group and survival group. $p<0.05$ was considered statistically significant.

Dynamic Changes of Lymphocyte Subsets in Patients with Severe COVID-19

In this study, we analyzed the dynamic changes of WBC, lymphocyte counts, as well as different lymphocyte subsets in patients with severe COVID-19 (Figure 2). The decrease of CD3+ T cells, CD4+ T cells, CD8+ T cells, and CD45+ T cells counts in the survival group reached its peak from hospital admission to 3 days after admission, and then gradually increased during the

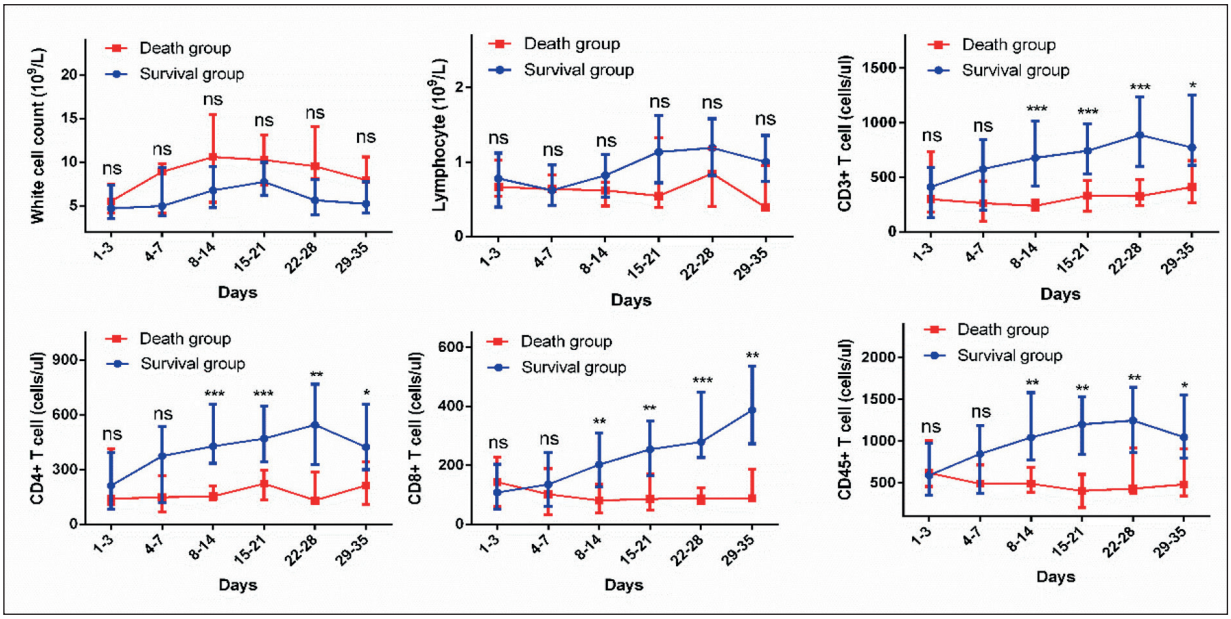

Figure 2. Kinetic analysis of T lymphocyte subsets in patients with severe COVID-19. The absolute numbers of CD3+ T cells, CD4+ T cells, CD8+ T cells, and CD45+ T cells in the survival group (blue line) and death group (red line) patients were analyzed at different time points after hospital admission. The levels of T cells are shown using median and interquartile range.

4 to 28 days after admission. In contrast, a sustained decrease in the counts of CD3+ T cells, CD4+ T cells, CD8+ T cells, and CD45+ T cells was observed in the death group during the follow up. The absolute numbers of CD3+ T cells, CD4+ T cells, CD8+ T cells, and CD45+ T cells were markedly lower in the death group compared with that in the survival group from 8 to 35 days after admission (all $p < 0.05$) (Table IV). No significant differences were observed in WBC and lymphocyte counts between the survival group and death group during the follow up (Figure 2).

Discussion

SARS-CoV-2 infection can cause acute respiratory syndrome with consequent release of pro-inflammatory cytokines. In severe COVID-19, acute respiratory distress is often accompanied by cytokine storm, which is characterized by an enormous secretion of pro-inflammatory cytokines[8]. SARS-CoV-2 infection with cytokine storm generally occurs in subjects with ARDS, and non-survival in ARDS was linked to sustained pro-inflammatory cytokines elevation. Additionally, T cells are important for dampening overactive innate immune responses during viral infection. A loss of T cells may result in aggravated inflammatory responses[9]. Therefore, it is necessary to study the dynamics of cytokines and T lymphocyte subsets of patients with severe COVID-19 and their correlation with the prognosis.

Chen et al[4] characterized the kinetic changes of inflammatory cytokine levels, including IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-a, at 16 days after disease onset, in 13 patients with severe COVID-19[5]. Another study demonstrated changes in inflammatory cytokine levels, such as IL-2, IL-6, IL-10, and TNF-a, in 11 patients with severe COVID-19. However, these studies have a small number of patients, short-term following up, and limited number of cytokines. In this study, we showed the dynamics of twelve cytokines from

Table IV. Comparison in the levels of lymphocyte subsets between death group and survival group.

		Death group (n=7)	Survival group (n=24)	*p*-value
CD3+ T cells (cells/ul)	day 1-3	300 (181-737)	411 (128-592)	0.910
	day 4-7	262 (100-466)	577 (200-845)	0.093
	day 8-14	239 (198-291)	679 (421-1018)	**<0.001**
	day 15-21	333 (189-472)	744 (531-992)	**<0.001**
	day 22-28	328 (240-480)	889 (602-1234)	**<0.001**
	day 29-35	412 (269-653)	773 (612-1252)	**0.014**
CD4+ T cells (cells/ul)	day 1-3	141 (109-413)	212 (82-394)	0.717
	day 4-7	148 (67-265)	374 (118-536)	0.077
	day 8-14	153 (132-210)	428 (334-660)	**<0.001**
	day 15-21	222 (136-297)	470 (343-648)	**<0.001**
	day 22-28	132 (112-287)	545 (327-767)	**0.003**
	day 29-35	117 (98-342)	423 (300-658)	**0.035**
CD8+ T cells (cells/ul)	day 1-3	143 (61-227)	108 (51-204)	0.540
	day 4-7	102 (33-189)	135 (61-244)	0.155
	day 8-14	81 (39-136)	202 (128-311)	**0.001**
	day 15-21	86 (49-170)	254 (164-350)	**0.005**
	day 22-28	88 (70-125)	279 (226-447)	**<0.001**
	day 29-35	89 (83-206)	387 (273-536)	**0.005**
CD45+ T cells (cells/ul)	day 1-3	618 (457-1010)	590 (347-980)	0.686
	day 4-7	488 (373-714)	847 (375-1190)	0.182
	day 8-14	488 (388-687)	1045 (777-1581)	**0.001**
	day 15-21	403 (203-601)	1201 (840-1530)	**0.001**
	day 22-28	431 (371-916)	1248 (861-1647)	**0.003**
	day 29-35	480 (345-906)	1049 (798-1554)	**0.014**

The *p*-values indicate differences between death group and survival group. $p<0.05$ was considered statistically significant.

hospital admission to 35 days after admission in 31 patients with severe COVID-19. Compared with previous studies, this study enrolled more patients, tested more cytokines, and showed longer follow-up period.

Previous studies[10,11] have shown that elevated levels of cytokines and decreased levels of lymphocyte subsets are associated with severe lung injury of patients with COVID-19. Hou et al[10] found that lymphopenia and increased levels of cytokines were closely associated with the disease severity of COVID-19. Zhang et al[11] reported that lymphocytopenia, especially the reduced CD4+ T cell and CD8+ T cell counts upon admission, were predictive of disease progression, and high levels of IL-6 and IL-8 during treatment were observed in patients with severe disease. However, these researches only reported that the inflammatory cytokines were related with the disease progression. The data about the dynamics of cytokines and lymphocyte subsets and their correlation with the clinical prognosis of severe COVID-19 are lacking. In this study, compared with the survival group, the death group had higher concentrations of IL-6, IL-8, and IL-10, and lower concentrations of T lymphocyte subsets, suggesting that the magnitude of cytokine storm and lymphopenia is associated with the clinical prognosis of severe COVID-19.

Conti et al[12] showed that the significant and sustained increases in the serum levels of IL-6, IL-8, and IL-10 were associated with the prognosis of severe COVID-19. IL-6 plays an important role in the pathology of COVID-19. Magro et al[13] reported that IL-6 has both pro and anti-inflammatory properties, depending on the pathway of transduction. The IL-6 inhibitor (tocilizumab) has been suggested for the treatment of severe COVID-19[14]. Our results showed that IL-6 is associated with the prognosis of severe COVID-19, which is consistent with previous reports[15,16]. Liu et al[15] reported that IL-6 could be used as independent factors to predict the severity of COVID-19, and patients with IL-6 > 32.1 pg/mL were more likely to have severe complications. Wan et al[16] found that higher survival rates occurred in patients with IL-6 within normal values. Aziz et al[17] performed a meta-analysis of 9 studies, and found that severe COVID-19 had significantly higher serum IL-6 compared to non-severe cases, and increasing mean IL-6 levels were associated with increased mortality in patients.

Chen et al[4] reported that SARS-CoV-2 infection primarily affected T lymphocytes particularly CD4+ T cells and CD8+ T cells. Grifoni et al[18] reported that CD4+ T cell responses to spike, the main target of most vaccine efforts, were robust and correlated with the magnitude of anti-SARS-CoV-2 IgG and IgA titers. Wang et al[19] reported that total lymphocytes, CD4+ T cells, and CD8+ T cells decreased in patients with COVID-19, and severe cases had a lower levels than mild cases. This study also found that the lymphopenia in severe COVID-19 was mainly related to the significantly decreased counts of T cells, especially CD4+ T cells and CD8+ T cells. The decrease of CD3+ T cells, CD4+ T cells, CD8+ T cells, and CD45+ T cells in the survival group reached its peak from hospital admission to 3 days after admission, and then gradually increased during the 4 to 28 days after admission.

Our study has some limitations. First, this study is a single-center, retrospective study. Second, the data regarding the viral load of SARS-CoV-2 are not available for patients with COVID-19 in this study. Further studies are needed to investigate the correlation between the viral load kinetics and the dynamics of cytokines and lymphocyte subsets.

Conclusions

In summary, we showed the dynamic changes of cytokines and lymphocyte subsets of severe COVID-19 and their correlation with the disease prognosis. Compared with the survival group, the death group showed significant and sustained increases in IL-6, IL-8, and IL-10, but decreases in CD3+ T cells, CD4+ T cells, CD8+ T cells, and CD45+ T cells counts. This study may help to achieve a better understanding of immune function disorder following SARS-CoV-2 infection and help physicians to provide timely intervention for severe COVID-19.

Declaration

All patients in this study have been included in a previous report named "A simple algorithm helps early identification of SARS-CoV-2 infection patients with severe progression tendency" (Li Q, Zhang J, Ling Y, Li W, Zhang X, Lu H, Chen L. A simple algorithm helps early identification of SARS-CoV-2 infection patients with severe progression tendency. Infection 2020; 48: 577-584).

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Authorship contributions

Study concept and design: Liang Chen. Data collection: Qiang Li, Wei Xu, Weixia Li, and Chenlu Huang. Analysis and interpretation of data: Qiang Li, Wei Xu, and Weixia Li. Drafting of the manuscript: Qiang Li, Wei Xu, and Weixia Li. Critical revision of the manuscript: Liang Chen.

Consent for publication

All authors read and approved the manuscript.

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Role of the sponsor

The funding organizations are public institutions and had no role in the design and conduct of the study; collection, management, and analysis of the data; or preparation, review, and approval of the manuscript.

Availability of data and materials

We declared that materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for non-commercial purposes, without breaching participant confidentiality. The supporting data can be accessed from Liang Chen (corresponding author), e-mail: chenliang@shphc.org.cn

Ethics approval and consent to participate

The verbal informed consents were obtained from all participants. The Ethics Committee of Shanghai Public Health Clinical Center approved the study protocol, and experiments, including any relevant details. This study was performed in accordance with the Declaration of Helsinki. All experiments were performed in accordance with relevant guidelines and regulations.

Conflict of Interests

The authors declare that they have no conflict of interest.

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