Thymosin alpha 1 (Tα1) reduces the mortality of severe COVID-19 by restoration of lymphocytopenia and reversion of exhausted T cells

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Summary: Thymosin alpha 1 (Tα1) reduces the mortality of severe COVID-19 by restoration of lymphocytopenia and reversion of exhausted T cells. COVID-19 patients with the counts of $CD8^+$ or $CD4^+$ T cells lower than 400/μL or 650/μL, respectively, gain more benefits.

Abstract

Background. We previously reported that lymphocytopenia and T cell exhaustion is notable in acute COVID19 patients, especially in aged and severe cases. Thymosin alpha 1 (Tα1) had been used in the treatment of viral infections as an immune response modifier for many years. However, clinical benefits and mechanism of Tα1 supplement to COVID-19 are still unclear.

Methods. We retrospectively reviewed the clinical outcomes of 76 severe cases with COVID-19 admitted into two hospitals in Wuhan from December 2019 to March 2020. The thymus output in peripheral blood mononuclear cells (PBMCs) from COVID-19 patients was measured by T cell receptor excision circles (TREC). The levels of T cell exhaustion markers PD-1 and Tim-3 on $CD8^+$ T cells were detected by flow cytometry.

Results. Compared with untreated group, Tα1 treatment significantly reduces mortality of severe COVID-19 patients (11.11% *vs.* 30.00%, p=0.044). Tα1 timely enhances blood T cell numbers in COVID-19 patients with severe lymphocytopenia (the counts of $CD8^+$ T cells or $CD4^+$ T cells in circulation lower than 400/μL or 650/μL, respectively). Under such conditions, Tα1 also successfully restores $CD8^+$ and $CD4^+$ T cell numbers in aged patients. Meanwhile, Tα1 reduces PD-1 and Tim-3 expression on $CD8^+$ T cells from severe COVID-19 patients in comparison with untreated cases. It is of note that restoration of lymphocytopenia and acute exhaustion of T cells are roughly parallel to the rise of TRECs.

Conclusions. Tα1 supplement significantly reduce mortality of severe COVID-19 patients. COVID-19 patients with the counts of $CD8^+$ T cells or $CD4^+$ T cells in circulation lower than 400/μL or 650/μL, respectively, gain more benefits from Tα1. Tα1 reverses T cell exhaustion and recovers immune reconstitution through promoting thymus output during SARS-CoV-2 infection.

Key Words: Thymosin alpha 1; COVID-19; SARS-CoV-2; T cell exhaustion; Immune reconstitution

Introduction

The epidemic of 2019 novel coronavirus (SARS-CoV-2), which was first reported in December 2019 in Wuhan, China, has been recognized as a pandemic by the World Health Organization (WHO) [1-3]. As of May 13, 2020, China has reported 84451 cases of confirmed COVID-19 and 4644 fatalities. Meanwhile, the number of confirmed COVID-19 and fatalities in the whole world were 4098018 and 283271, respectively [4].

Lymphocytes play an essential role in fighting against viral infections, therefore, boosting the number and enhancing the antiviral function of T cells in COVID-19 patients is of paramount value for successful recovery. However, most COVID-19 cases displayed severe lymphocytopenia, especially in aged and severe cases [2, 5-7]. Thymosin-α1 (Tα1), a kind of polypeptide hormone produced by thymic epithelial cells, can effectively increase T cell numbers, support T cell differentiation, maturation, and reduce cell apoptosis [8-10]. Therefore, Tα1 has been successfully used in clinical practice to treat patients infected with hepatitis B (HBV), hepatitis C (HCV) and human immunodeficiency viruses (HIV), and its efficacy was proved by pathological observation [11-13]. To enhance immunity, actually, the medical support team members from all over the country got Tα1 injection before being deployed to Hubei Province, and no infectious cases were reported till now, suggesting Tα1 might have the potential to prevent SARS-CoV-2 infection. Nevertheless, no data are available whether Tα1 treatment has any benefits to critically ill COVID-19 patients yet.

We here retrospectively analyzed the clinical data from 76 critically ill cases of COVID-19 who were admitted into General Hospital of the Central Theatre Command and Wuhan Pulmonary Hospital in Wuhan from December 2019 to March 2020. We also compared the expression of exhaustion markers PD-1 and Tim-3 on $CD8^+$ T cells and analyzed thymus output ability from COVID-19 patients after Tα1 treatment. Our results provide a preliminary demonstration that Tα1 has benefits to COVID-19 patients, especially those with severe lymphocytopenia.

Methods

Patients Medical records of severe/critical COVID-19 patients (aged from 21 years to 92 years) admitted into General Hospital of the Central Theatre Command or Wuhan Pulmonary Hospital in Wuhan were collected and retrospectively analyzed. Diagnosis and classification of clinical types of COVID-19 were based on the New Coronavirus Pneumonia Prevention and Control Program (5th edition) published by the National Health Commission of China. To be brief, patient who meets any of the following conditions is diagnosed as severe type: patients present respiratory distress with

respiratory rate ≥ 30 breath/min, SpO2 (oxygen saturation) ≤ 93% on room air, and PaO2 (arterial blood oxygen partial pressure)/FiO2 (fraction of inspired oxygen) ≤ 300 mmHg (1 mmHg = 0.133 kPa); patient meets any of the following conditions is diagnosed as critical type: patient presents respiratory failure and requires mechanical ventilation support, patient presents shock, and patient presents multiple organ dysfunction syndrome and requires ICU admission. Only severe or critical COVID-19 patients with at least 10 days' hospitalization duration were finally included in this study. According to the presence or absence of 7 days continuous Tα1 injection, the enrolled patients under study were categorized as treatment group and non-treatment group. The endpoint of this study was recovery or death.

Tα1 management In the treatment group, patients received subcutaneous injections of 10 mg Tα1 once per day for at least seven consecutive days, then still recommended to continue to use till the endpoints of the present study. Prior to administration, the lyophilized powder is to be reconstituted with 1 ml of the provided diluent (sterile water for injection). After reconstitution, the final concentration of Ta1 is 10 mg/ml. The workflow chart was showed in **Figure 1**.

Data collection We reviewed clinical records, nursing records, laboratory findings, and chest x-rays or CT scans for all the patients. All information was obtained and curated with a customized data collection form. Two investigators (Y Liu and Y Chen) independently reviewed the data collection forms to verify data accuracy.

PD-1 and Tim-3 Assay Peripheral blood samples from patients were harvested with anticoagulants (EDTA-K_2). Lymphocytes were gated from $CD45^+$ leukocytes with using anti-CD45-APC antibodies (SK1, BD Biosciences), the expression of exhaustion markers on $CD8^+$ T cells were further detected with using CD8-PE (SK1, Biolegend), PD-1-PE-CY5 (EH12.2H7, Biolegend) and TIM-3-FITC (F38-2E2, Biolegend) antibodies. After being stained, the cells were measured by flow cytometry on an LSR Fortessa Cell Analyzer (BD Biosciences) and data analyzed using the FolwJo software (TreeStar). All experimental procedures were completed under biosafety level II plus condition.

TREC assay Peripheral blood samples from patients were harvested with anticoagulants (EDTA-K_2). Peripheral blood mononuclear cells (PBMCs) were harvested by density gradient centrifugation in the Central Lab of General Hospital of the Central Theatre Command. TREC assay was performed using DNA extracted from the participants' PBMCs. Amplification reactions (20μl) contained approximate 20 ng of genomic DNA, 10 μl of 2 × Premix Ex Taq master mix (Takara Bio Inc. Japna), and the appropriate primers and probes. PCR conditions including primers and probe sequences have been described previously [14]. Reactions were carried out in an Roche LightCycler®96 detection system (Roche Applied Science, Germany). The copies of TRECs in a

given sample was estimated by comparing the CT value with a standard curve obtained from PCRs performed with tenfold serial dilutions of an internal standard. Amplification of RNAseP (Applied Biosystems) was used to verify the quantity and presence of genomic DNA. TREC values were adjusted for total DNA content.

Results

1. Tα1 reduces COVID-19 patient mortality

To fight against virus infection, it is necessary to boost T cell numbers and their antiviral functions in COVID-19 patients due to no specific drugs for SARS-CoV-2 coronavirus are available till now. Tα1, a kind of polypeptide hormone that can regulate T cell production, differentiation and activity, was selected to treat COVID-19 patients. A total of 76 severe or critical COVID-19 from two designated hospitals were enrolled in this retrospective cohort study based on the pre-set inclusion criteria. According to the presence or absence of 7 days continuous Tα1 injection, the patients under study were categorized as treatment group in 36 patients and non-treatment group in 40 patients. There were no significant differences between the two groups in demographic characteristics and clinical findings at the time of admission, such as age, sex, coexisting disorders, the counts of total lymphocytes, T cells, $CD8^+$ T cells, $CD4^+$ T cells, PCO_2 on admission and PCO_2 of discharge. Moreover, all of these patients got antiviral and antibacterial treatment during hospitalization. Glucocorticoid and oxygen inhalation are also very common. Tα1-treated group has slightly higher level of IL-6 than untreated group (p=0.047), whereas, the levels of PO2 on admission and PO2 of discharge are a little higher in Tα1- treated group than those in non-treatment group (**Table 1**). Ta1 has been approved by FDA and China FDA, and it is a very well tolerated drug. No side effects associated with Ta1 were found after thorough reading of all the medical records of 36 participants in the treatment group. There were 2 patients in treatment group underwent noninvasive mechanical ventilation while 11 patients underwent in non-treatment group. 9 out of the 11 cases in the control arm underwent subsequent invasive mechanical ventilation owing to hypoxia cannot by adjusted by noninvasive mechanical ventilation whereas none in the treatment arm were intubated. More interestingly, 11.11% (4/36) cases with Tα1 treatment succumbed, whereas, the mortality of severe COVID-19 patients without Tα1 treatment reaches 30% (12/40) (**Figure 2, Table 1)**, suggesting Tα1 supplement can reduce mortality.

2. Tα1 enhances T cell counts in COVID-19 patients with severe lymphocytopenia

We next investigated whether Tα1 can restore T cell numbers in COVID-19 patients with severe

lymphocytopenia. 34 cases with recording of T cell numbers on admission and after continuous 7 days of Tα1 treatment were involved in this section. Results showed that Tα1 supplement effectively restores T cell numbers in cases with the counts of $CD8^+$ T cells or $CD4^+$ T cells lower than 400/μL or 650/μL, respectively, whereas, patients whose T cell numbers are higher than the above-mentioned levels gain no benefits from Tα1 treatment (**Figure 3A**). More interestingly, Tα1 also dramatically increases the counts of $CD8^+$ and $CD4^+$ T cells in aged patients (> 60 years old) (**Figure 3B**), and cases with comorbidities of hypertension or cardiovascular diseases (**Figure 3C**). Collectively, these data demonstrate that Tα1 promotes timely T cell recovery in COVID-19 patients with severe lymphocytopenia.

3. Tα1 reveres $CD8^+$ T cell exhaustion *via* enhancing thymus output

Our previous studies have demonstrated that SARS-CoV-2 infection induces acute T cell exhaustion, which might lead to ineffectively eliminate viruses *in vivo* [7]. We then analyzed whether Tα1 affects T cell exhaustion in severe COVID-19 patients, hence the expression of programmed death-1 (PD-1) and T cell immunoglobulin and mucin domain protein 3 (Tim-3) on $CD8^+$ T cells was detected by flow cytometry. Because of retrospective nature of this study, peripheral blood samples from 22 enrolled patients, including 10 patients from Non-treatment group and 12 patients from Tα1-treatment group respectively, were available at the time of performing PD-1/Tim-3 analysis. Results showed that Tα1 effectively down-regulates both PD-1 and Tim-3 on $CD8^+$ T cells in cases with Tα1 treatment than those in non-treatment group (**Figure 4A**), suggesting that Tα1 can partially reverse T cell exhaustion during SARS-CoV-2 infection.

We next investigated whether the reversion of $CD8^+$ T cell exhaustion in COVID-19 patients by Tα1 is due to enhancement of thymus-derived naïve T cells in circulation. TRECs are specific circular DNA byproducts formed during the random rearrangements of T cell receptors, and they are only present in cells exported from thymus but not found in replicating cells within PBMCs [15]. To explore whether Tα1 administration affects thymus output in COVID-19 patients, we measured TRECs in PBMCs of patients before and after 7 days' Tα1 injection. Interestingly, the Tα1 treated groups manifested significantly higher TREC levels than untreated control (**Figure 4B**), suggesting Tα1 has the capacity to enhance thymus output, by thus enhance TCR diversity and finally reverse $CD8^+$ T cell exhaustion.

Discussion

We here retrospectively reviewed and found that Tα1 significantly reduces mortality of severe COVID-19 patients compared with those in untreated group (**Figure 2**). Further study showed that

Tα1 effectively and timely enhances T cell counts in COVID-19 patients with severe lymphocytopenia, especially in cases with the counts of $CD8^+$ or $CD4^+$ T cells lower than 400/μL or 650/μL, respectively (**Figure 3A**). Based on these results, we strongly recommend COVID-19 patients whose $CD8^+$ T or $CD4^+$ T cell counts lower than 400/μL or 650/μL, respectively, apply Tα1 injection to improve their immune function. Additionally, the duration of Tα1 injection should be at least 7 days. Importantly, enhancement of T cells was also observed in aged patients and cases with coexisting disorders including hypertension and cardiovascular disease after 7 days continuous Tα1 supplement (**Figure 3B～D**). We therefore suggest healthy people aged above 60 receive Tα1 supplement to prevent potential SARS-CoV-2 infection due to the immune system of the elderly also have responses to Tα1, even the thymus might be extremely atrophy.

Immune damage is very common in COVID-19 patients, and most critically ill cases manifested severe lymphocytopenia [6, 16]. Tα1 has been described to regulate T cell development and enhance T cell numbers, it has also been used in many clinical settings in which T cell immunity is involved, including aging, viral infectious diseases, autoimmune disorders, and immune reconstitution after bone marrow transplantation [17-19]. Tα1 also showed promise in larger clinical studies of acute infections. For examples, several clinical studies demonstrated Tα1 has benefit in the treatment of severe sepsis, which begins with a bacterial or fungal infection [20]. Interestingly, Tα1 treatment also significantly increased the number of $CD4^+$ and $CD8^+$ cells in patients with CMV infection accompanied with acute respiratory distress syndrome (ARDS) after renal transplantation [21]. Similarly, we here extend this field and found that Tα1 supplement effectively boosts both $CD4^+$ and $CD8^+$ T cell numbers in COVID-19 cases with severe lymphocytopenia.

The naive $CD8^+$ T cells undergo robust proliferation and clonal expansion to differentiate into effector $CD8^+$ T cells that directly kill target cells during acute infection, $CD4^+$ T cells, on the other hand, majorly assist B cells to produce antibodies thus finally clear pathogen. However, some $CD8^+$ T cells are loss of effector functions and become exhausted during chronic infections and cancer where antigen stimulation persists, nevertheless, revitalization of exhausted T cells can reinvigorate immunity. The exhausted T cells express elevated inhibitory receptors like PD-1 or TIM-3 [22]. Interestingly, recent work also confirmed that exhausted T cells are existent in acute viral infections, and T cells from patients in the acute phase of Ebola infection manifested with enhancing PD-1 expression but impaired IFN-γ production [23]. Moreover, blocking Tim-3 signal efficiently enhanced IFN-γ secretion from T cells following H1N1 infection model [24]. Our previous work has firstly reported that SARS-CoV-2 triggers the expression of PD-1 and Tim-3 on T cells, suggesting exhausted T cells were persistent in COVID-19 patients during SARS-CoV-2 acute

infection [7]. **W**e here found that Tα1 effectively down-regulates both PD-1 and Tim-3 on $CD8^+$ T cells in COVID-19 patients (**Figure 4A**), suggesting Tα1 might also boost immune response in hosts through revering T cell exhaustion during acute viral infection.

Inflammation would promote exhausted T cell differentiation. For example, IL-6, a pleiotropic cytokine that plays an essential role in regulating immune responses, can rescue lymphocyte from exhaustion following HCV infection [25]. IL-10 is an inhibitory cytokine that has the capacity to induce T cell exhaustion, and blocking IL-10 function has been shown to successfully prevent T cell exhaustion following chronic LCMV infection [26, 27]. The severe/critically ill COVID-19 patients have very high levels of serum IL-6 and IL-10, and these patients also displayed high levels of the PD-1 and Tim-3 on T cells, suggesting that both IL-6 and IL-10 might be mechanistically responsible for mediating exhausted T cell differentiation in COVID-19 patients [7]. Moreover, serum levels of other inflammation factors including PCP, CRP and ferritin on admission were also very high in COVID-19 patients (**Table 1**). However, due to the outbreak of SARS-CoV-2 infection is extremely emergency in February, we regret having not the capacity to measure serum IL-10, IL-6 and inflammation markers including PCP, CRP and ferritin in Tα1-treated patients. Further work is needed to clarify the exact mechanism underlying Tα1 down-regulates PD-1 and Tim-3 expression on $CD8^+$ T cells.

The number of lymphocytes from thymus will decrease in aged person due to thymus dysregulation and atrophy, so TCR repertoire diversity of lymphocytes in the peripheral blood of the elderly are decreased, which might make the elderly more susceptible to virus infection [28-30]. This phenomenon was also seen in severe COVID-19 patients due to overwhelming cases in ICU are aged above 60 [1, 2, 31]. Actually, altered T cell repertoire diversity occurs in exhausted T cells [32], and reduces thymic output might be a major mechanism of immune reconstitution failure in HIV-infected patients after long-term antiretroviral therapy [33]. In this study, we found that Tα1 can promote thymus output in COVID-19 patients based on the levels of TREC (**Figure 4B**), which is an accurate method to detect thymus output in circulation T cells [34], demonstrating Tα1 enhances immune reconstitutions through promoting thymus output leading to enhancement of TCR repertoire diversity and lymphocyte numbers in circulation.

However, we acknowledge that our study has several limitations. One issue is the normalization of TREC levels among individuals, there are no specific guidelines as to how to normalize circulating TREC copies, and we chose to adjust to total DNA content in the sample. The second issue is we only investigated PD-1 and Tim-3 on $CD8^+$ T cells, further studies using peptides from SARS-CoV-2 virus to activate T cell *in vitro* and clarify whether Tα1 treatment also can enhance IL-2 and IFN-γ secretion from peptides- activated T cells is needed. The third issue is no detective

kits are available to quantify virus titer after Tα1 treatment, so we did not know whether Tα1 treatment can control virus titers till now. Fourthly, although our results show Tα1 treatment has some benefits to COVID-19 patients, it should be interpreted with caution because of inherent nature of retrospective study and small sample size, future longitudinal studies on a larger cohort are eagerly needed. Fifthly, it's very difficult to retrieve clinical improvement related index, which is why we just chose mortality as our primary clinical outcome and is very unlike a recent RCT research [35], in that study a seven-category ordinal scale was designed prior to the commence of research. Additionally, mortality of this study was crude mortality, which included both COVID-19-attributable mortality and mortality related to underlying disease. 4 out of the 36 cases receiving Tα1 treatment succumbed, accounting for 11.11%. Some serious complications occurred in these 4 cases during clinical courses of COVID-19, such as hemorrhagic shock resulting from gastrointestinal hemorrhage in case 1, heart attack in case 2, septic shock in both case 3 and case 4. It is very difficult to tell which exactly contributes to their corresponding deaths. Last but not the least, the characteristics of enrolled patients at baseline were generally matched between the two groups, however, some confounders during clinical courses, including but not limited to glucocorticoids dosage or exposure levels, are inevitably to avoid and difficult to evaluate.

In conclusion, we here demonstrated that Tα1 supplement has the capacity to improve and restore T cell counts in COVID-19 patients with severe lymphocytopenia. Importantly, Tα1 supplement can reverse T cell exhaustion and induces immune reconstitution through inducing thymus output in COVID-19 patients with SARS-CoV-2 infection. All of these have collectively contributed to the reduction in mortality in this study cohort. Though our results should be interpreted with caution, it does give a clue to the treatment of COVID-19 patients and future similar studies.

Authors' contributions

Bo Diao, Yuzhang Wu, and Yongwen Chen involved in the final development of the project and manuscript preparation; Zhenhong Hu, Zilin Yuan, Yue Pang, Chenghui Wang and Zeqing Feng analyzed the data; Bo Diao and Ying Liu and Yueping Liu performed TRECs; Congzheng Mao, Yingjun Tan and Li Chen conducted Serum ELISA; Min Li and Gang Wang did flow cytometry analysis.

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This study was approved by the Ethics Commission of General Hospital of the Central Theatre Command and Wuhan Pulmonary Hospital. Written informed consent was waived by the Ethics Commission of the designated hospital for emerging infectious diseases and anonymous analysis of data.

Disclaimer

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

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Figure legends

Figure 1 Workflow chart. Tα1 supplement in combination with standard or conventional medical therapies is defined as treatment group while just standard or conventional medical therapies are defined as control group.

Figure 2 The survival rate (A), noninvasive mechanical ventilation (Free-%) (B) and invasive mechanical ventilation (Free-%) (C) of severe or critical COVID-19 patients with or without Tα1 supplement. There were 2 patients in treatment group underwent noninvasive mechanical ventilation while 11 patients underwent in non-treatment group. 9 out of the 11 cases underwent invasive mechanical ventilation owing to hypoxia cannot by adjusted by noninvasive mechanical ventilation. *p< 0.05. NMV: noninvasive mechanical ventilation; IMV: invasive mechanical ventilation.

Figure 3 Tα1 significantly enhances T cell counts in COVID-19 patients with severe lymphocytopenia. (**A**) Thymosin supplement effectively restores T cell numbers in cases with the counts of $CD8^+$ T cells or $CD4^+$ T cells lower than 400/μL or 650/μL, respectively; (**B**) Tα1 dramatically increases the counts of $CD8^+$ and $CD4^+$ T cells in aged patients (> 60 years old); Enhancement of T cell numbers by Tα1 supplement was also observed in cases with comorbidities of hypertension (**C**) or cardiovascular diseases (**D**). *p < 0.05; **p < 0.01; ***p < 0.001. NS: not significantly different.

Figure 4 Tα1 supplement enhances thymus output and reveres T cell exhaustion in severe COVID-19 patients. (**A**) The percentage of PD-1 and Tim-3 on $CD8^+$ T cells in severe COVID-19 patients without or with continuous 7 days of Tα1 supplement. (**B**) TREC levels in PBMCs of severe COVID-19 patients before and after 7 days of Tα1 treatment. **p < 0.01; ****p < 0.0001.

Table 1 Clinical Characteristics of severe/critically ill COVID-19 patients with or without Tα1 supplement. According to the presence or absence of 7 days continuous Tα1 injection, the patients under study were categorized as treatment group in 36 patients and non-treatment group in 40 patients.

	Treatment Group (N=36)	Non-treatment Group (N=40)	p value
Thymosin Treatment Time (Days)	17.5 (14 - 27.75)	NA *	
Age (Years)	56 (41.3 - 69.8)	57.5 (53.50 - 73.75)	0.3620
Male (%)	69.44 (25/36)	50 (20/40)	0.0850
Coexisting Disorders (%)	38.89 (14/36)	42.5 (17/40)	0.7494
Mortality (%)	11.11 (4/36)	30 (12/40)	0.0437
ICU length of stay of survivors (Days) (N = 32 vs. 27)	6.5 (4.25 - 10.75)	4 (1 - 14.0)	0.2300
Hospital stay of survivors (Days) (N = 32 vs. 28)	22.5 (16.85 - 34.25)	20.4 (18.76 - 26.31)	0.3140
Total T cells on admission (*10^6/L) (N = 34 vs. 20)	632.5 (365 - 812)	612 (293 - 461)	0.7880
CD8+ T cells on admission (*10^6/L) (N = 34 vs. 20)	208 (95 - 298)	190.5 (118 - 280)	0.9860
CD4+ T cells on admission (*10^6/L) (N = 34 vs. 20)	330 (175 - 411)	320 (132 - 597)	0.6800
Ratio of total T cells of discharge to admission (N = 21 vs. 20) (%)	1.65 (1.23 - 2.32)	3.78 (3.65 - 5.27)	0.1410
Ratio of CD8+ T cells of discharge to admission (N = 21 vs. 20) (%)	1.68 (1.23 - 2.18)	3.05 (2.50 - 4.44)	0.1030
Ratio of CD4+ T cells of discharge to admission (N=21 vs. 20) (%)	1.5 (1.28 - 1.50)	4.13 (3.30 - 9.70)	0.1200
Neutrophil on admission (*10^9/L)	3.95 (2.54 – 5.31)	3.95 (2.55 – 6.36)	0.818
Lymphocytes of admission (*10^9/L)	0.97 (0.64 - 1.35)	0.73 (0.46 - 0.70)	0.0770
Neutrophil-Lymphocyte ratio on admission	3.88 (2.54 – 7.29)	4.37 (2.74 – 9.83)	0.657
IL-6 on admission (pg/ml) (N = 36 vs. 33)	24 (17.13 - 48.48)	10.4 (7.30 - 37.25)	0.0470
PCT on admission (ng/mL) (N = 36 vs. 33)	0.07 (0.05 – 0.115)	0.06 (0.04 – 0.125)	0.838
CRP on admission (mg/L) (N = 36 vs. 39)	15.95 (8.95 – 48.37)	19.55 (13.96 – 65.45)	0.560
Ferritin on admission (ng/mL) (N = 11 vs. 12)	890.8 (437.4 – 1707.0)	472.4 (254.4 – 1016.1)	0.684
PO2 on admission (N = 25 vs. 17) (Kpa)	10.7 (9.75 - 15.3)	9.33 (7.74 - 13.32)	0.0320
PO2 of discharge (N = 20 vs. 18) (Kpa)	12.6 (9.77 - 16.68)	9.7 (6.57 - 12.35)	0.0420
Ratio of PO2 of discharge to admission (N = 20 vs. 17) (%)	1.1 (0.96 - 1.28)	0.95 (0.76 - 0.95)	0.1700
Time from admission to measurement of PO2 (Days) (N = 20 vs. 17)	8 (5.00 -18.50)	8 (5.50 - 9.00)	0.3740
Antiviral treatment (%)	100 (36/36)	100 (40/40)	1.0000
Oseltamivir (%)	88.9 (32/36)	87.5 (35/40)	0.852
Lopinavir/Ritonavir (%)	50 (18/36)	40 (16/40)	0.381
Ribavirin (%)	50 (18/36)	40 (16/40)	0.381
IFN-α (%)	58.3 (21/36)	47.5 (19/40)	0.345
Arbidol (%)	5.6 (2/36)	7.5 (3/40)	0.733
TCM (%)	91.7 (33/36)	80 (32/40)	0.149
Antibacterial treatment (%)	100 (36/36)	100 (40/40)	1.0000
Moxifloxacin (%)	75.0 (27/36)	62.5 (25/40)	0.242
Ceftriaxone (%)	58.3 (21/36)	22.5 (9/40)	0.001
Cefoperazone sulbactam (%)	52.8 (19/36)	65.05 (26/40)	0.279
Meropenem (%)	52.8 (19/36)	25.0 (10/40)	0.013

Levofloxacin (%)	8.3 (3/36)	27.5 (11/40)	0.031
Azithromycin (%)	5.6 (2/36)	7.5 (3/40)	0.733
Oxygen inhalation (%)	83.33 (30/36)	95 (38/40)	0.0980
Glucocorticoid (%)	66.67 (24/36)	95 (38/40)	0.0015
Intravenous (%)	100 (24/24)	100% (38/38)	1.000
Duration of corticosteroid treatment (days),	7 (4.25 – 16.75)	5 (4.00 – 8.00)	0.121
Total dose of methylprednisolone (mg)	885 (205 – 1390)	400 (260 – 585)	0.076
Dose of methylprednisolone per day (mg)	81.18 (57.28 – 116.69)	68.57 (50.29 – 91.66)	0.326
Low-to-moderate doses of corticosteroids (%) (25-150 mg/d methylprednisolone or equivalent)	91.7 (22/24)	92.1 (35/38)	0.951
High-dose corticosteroid therapy (%) (> 150 mg/d methylprednisolone or equivalent)	8.3 (2/24)	7.9 (3/38)	0.951
Noninvasive mechanical ventilation (%)	5.56 (2/36)	27.5 (11/40)	0.0198
Invasive mechanical ventilation (%)	0 (0/36)	22.5 (9/40)	0.0027
Blood purification (%)	2.78 (1/36)	0 (0/40)	0.2886

NA: Not applicable. NMV: noninvasive mechanical ventilation; IMV: invasive mechanical ventilation. TCM: Traditional Chinese Medicine, mainly including Lianhuaqingwen Capsule and Ganmao Qingre Granules.

Data are median (interquartile range, IQR) or % (n/N), where N is the total number of patients with available data. p values are from $\chi 2$ test or Mann-Whitney U test.

Figure 1

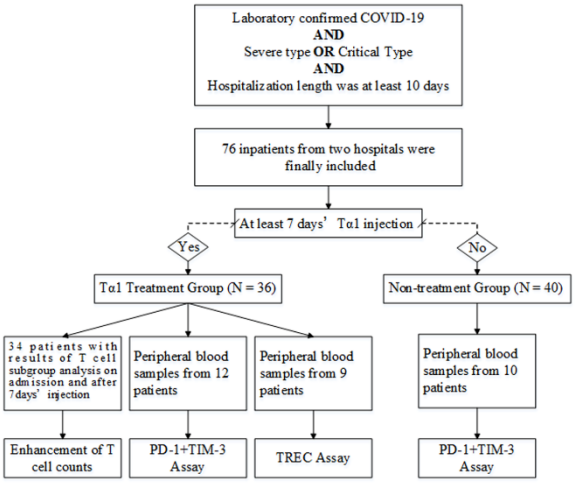

Figure 2

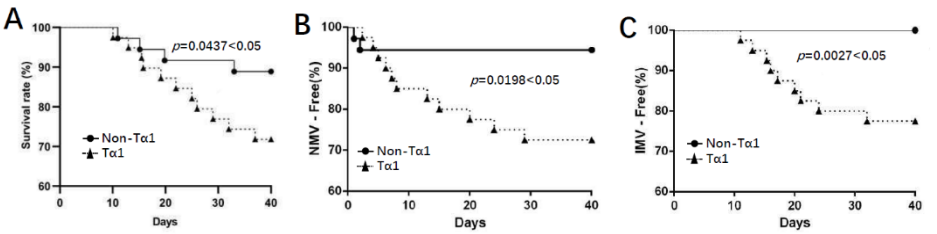

Figure 3

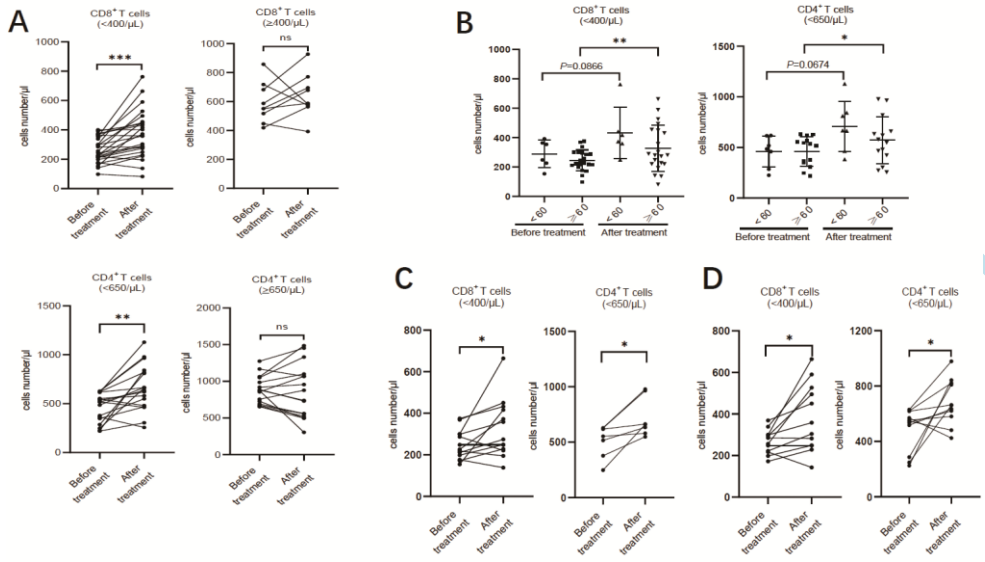

Figure 4