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Thymosin α1 therapy in critically ill patients with COVID-19: A multicenter retrospective cohort study

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ABSTRACT

Background: COVID-19 characterized by refractory hypoxemia increases patient mortality because of immunosuppression effects. This study aimed to evaluate the efficacy of immunomodulatory with thymosin α1 for critical COVID-19 patients.

Methods: This multicenter retrospective cohort study was performed in 8 government-designated treatment centers for COVID-19 patients in China from Dec. 2019 to Mar. 2020. Thymosin α1 was administrated with 1.6 mg qd or q12 h for > 5 days. The primary outcomes were the 28-day and 60-day mortality, the secondary outcomes were hospital length of stay and the total duration of the disease. Subgroup analysis was carried out according to clinical classification.

Results: Of the 334 enrolled COVID-19 patients, 42 (12.6%) died within 28 days, and 55 (16.5%) died within 60 days of hospitalization. There was a significant difference in the 28-day mortality between the thymosin α1 and non-thymosin α1-treated groups in adjusted model ($P = 0.016$), without obvious differences in the 60-day mortality and survival time in the overall cohort ($P > 0.05$). In the subgroup analysis, it was found that thymosin α1 therapy significantly reduced 28-day mortality (Hazards Ratios HR, 0.11, 95% confidence interval CI 0.02–0.63, $P = 0.013$) via improvement of PaO_2/FiO_2 ($P = 0.036$) and prolonged the hospital length of stay ($P = 0.024$) as well as the total duration of the disease ($P = 0.001$) in the critical type patients, especially those aged over 64 years, with white blood cell $> 6.8 \times 10^9$/L, neutrophil $> 5.3 \times 10^9$/L, lymphocyte $< 0.73 \times 10^9$/L, $PaO_2/FiO_2 < 196$, SOFA > 3, and acute physiology and chronic health evaluation (APACHE) II > 7.

Conclusion: These results suggest that treatment with thymosin α1 can markedly decrease 28-day mortality and attenuate acute lung injury in critical type COVID-19 patients.

1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has been a critical threat to global health. Critical COVID-19 patients account for approximately 10–20% of all patients and are characterized by refractory hypoxemia caused by acute respiratory distress syndrome (ARDS). The mortality of critical COVID-19 patients could range from 22 to 78% [1]. However, well-established treatment and control options appear to be lacking, while current clinical treatment strategies for critical COVID-19 patients mainly include antiviral and oxygen therapy, as well as organ support [2,3]. Lu *et al.* found that SARS-CoV-2 contained a

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similar receptor-binding domain structure as severe acute respiratory syndrome coronavirus (SARS-CoV) by homology modelling [4], and COVID-19 patients might share some similar pathological characteristics with other severe coronavirus-related pneumonia patients, such as cytokine storm syndrome and lymphocytopenia [5]. SARS-CoV-2 infection can result in the activation of innate and adaptive immune cells in the host, and immune system dysfunction is related to poor prognosis [6,7]. Despite these observations, therapy-targeted immune system modulation is still unestablished for the treatment of COVID-19. Thymosin α1 is a peptide originally isolated from thymic tissue that was shown to restore immune function in thymectomized mice [8], with a dual mechanism during inflammation [9]. Thymosin α1 could restore the T cells by enhancing their maturation and inhibiting apoptosis [10,11]. In addition, it also could prevent a proinflammatory cytokine storm by increasing regulatory T cells [12]. As an immune modulator, thymosin α1 exerts great biological influence in regulating the function of the immune system in many diseases, including sepsis, chemotherapy-induced immunosuppression, and acquired immune deficiency syndrome [13].

There are currently no available data regarding the clinical efficiency of thymosin α1 in critical COVID-19 patients. The present study aimed to evaluate the potential therapeutic efficacy of thymosin α1 in critical COVID-19 patients. We retrospectively collected clinical data, including thymosin α1 treatment records and outcomes of critical COVID-19 patients from 8 centers in China. This study might provide information on the clinical application of thymosin α1 in the treatment of SARS-CoV-2 infection, especially in targeted population selection.

2. Materials and methods

2.1. Study design and participants

This multicenter retrospective cohort study was performed in 8 government-designated treatment centers for COVID-19 patients (4 intensive care units (ICUs) and 4 general wards) in 3 cities in China: Wuhan, Guangzhou, and Shenzhen. The data collection period was from December 2019 to March 2020, and the data cutoff date was April 3, 2020.

The following inclusion criteria were used: (1) adult aged ≥ 18 years old; (2) laboratory-confirmed (reverse transcription polymerase chain reaction, RT-PCR) SARS-COV-2 infection from throat swab, sputum and/or lower respiratory tract samples or confirmed plasma positivity for specific antibody (IgM or/and IgG) against SARS-COV-2; (3) in-hospital treatment ≥ 72 h (h); (4) any one of the following criteria for severe type (a-c) or criteria for critical type (d-f): (a) respiratory rate ≥ 30/min, (b) rest SPO_2 ≤ 90%, (c) PaO_2/FiO_2 ≤ 300 mmHg, (d) respiratory failure requiring mechanical ventilation, (e) occurrence of shock, or (f) multiple organ failure requiring ICU monitoring. The following exclusion criteria were used: women who are pregnant or breastfeeding.

2.2. Procedures

We designed the data collection form, and demographic, clinical, treatment, laboratory data and prognosis data were extracted from electronic medical records. Detailed clinical data from before and after prescription of thymosin α1 and the data at the corresponding time of the same period in the non-thymosin α1 group were collected. Prescription status, timing, dosages (1.6 mg, qd or q12 h), and duration of thymosin α1 were decided by the doctors in charge according to the Chinese Recommendations for Diagnosis and Treatment of Novel Coronavirus (SARS-CoV-2) Infection (Trial 7th Version) published by the National Health Commission of China. Comparisons were conducted according to whether thymosin α1 was used. The primary endpoints were the 28-day and 60-day mortality rates, and the secondary outcomes were the hospital length of stay and the total duration of the disease. The risk factors for 28-day mortality were estimated by the Cox proportional hazards model. Analysis of the outcomes and survival curves were carried out according to the clinical classification of COVID-19. The study was approved by the Research Ethics Commission of General Hospital of Southern Theater Command of PLA (HE-2020-08), and the requirement for informed consent was waived by the Ethics Committee.

2.3. Definitions

"Critical COVID-19" in the current study was defined as a combination of "severe type" and "critical type" COVID-19, classified according to the Chinese Recommendations for the Diagnosis and Treatment of Novel Coronavirus (SARS CoV2) Infection (Trial 7th version) published by the National Health Commission of China. Thymosin α1 administered via subcutaneous injection was a purified sterile lyophilized preparation of chemically synthesized thymosin α1, which is an acetylated polypeptide with the following sequence: Ac-Ser-Asp-Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu-Ile-Thr-Thr-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-Val-Val-Glu-Glu-Ala-Glu-Asn-OH, with a molecular weight of 3108 Da. The lyophilized preparation contained 1.6 mg thymosin α1, 50 mg mannitol, and sodium phosphate buffer to adjust the pH to 6.8. Prior to administration, the lyophilized powder was reconstituted with 1 ml of the provided diluent (sterile water for injection). After reconstitution, the final concentration of thymalfasin was 1.6 mg/ml.

2.4. Statistical analysis

The categorical data are summarized as numbers and percentages, and intergroup comparisons were performed using Mann-Whitney U, χ^2 tests or Fisher's exact test. Continuous variables are expressed as the arithmetic mean and standard deviation (SD) or as the median and interquartile range (IQR), depending on whether they showed a Gaussian distribution. Continuous data with a Gaussian distribution were compared with Student's *t* test or one-way ANOVA, and those with a non-Gaussian distribution were compared with the Wilcoxon rank-sum test. To determine the independent effect of 28-day mortality in critical COVID-19 patients after accounting for significant confounders, the Cox proportional hazards model was used with a fully adjusted model: hazards ratios (HRs) and 95% confidence intervals (95% CIs) were obtained. Moreover, for analysis of the 28-day and 60-day mortality rates, Kaplan-Meier survival curves and the log-rank test were used. Statistical analyses were performed using the SPSS Windows version 22.0 (SPSS Inc, Chicago, IL), and Empower (R) (http://www.empowerstats.com, X&Y solutions, Inc., Boston, MA) and R (http://www.R-project.org) software. *P* values (two-tailed) below 0.05 were considered statistically significant.

3. Results

3.1. Demographics and baseline characteristics

Clinical data of 334 patients with confirmed critical COVID-19 were collected. The detailed demographic and clinical profile data of all critically ill patients with COVID-19 at baseline are summarized in Table 1. The patients' mean age was 57 (IQR 45.0–67.0) years, and the mean body temperature was 37.0 °C (IQR 36.5–37.8). A total of 158 (47.3%) of the patients had comorbidities, mainly hypertension (100, 29.9%), diabetes (38, 11.4%), coronary heart disease (31, 9.3%), and chronic obstructive pulmonary disease (10, 3.0%). Of the 334 patients with critical COVID-19, 231 (69.2%) were severe type, 103 (30.8%) were critical type, 102 used thymosin α1 and 232 did not. In comparison to the non-thymosin α1 group, the disease was more severe in the thymosin α1 group, and the group was characterized by older age; higher acute physiology and chronic health evaluation (APACHE) II scores and sequential organ failure assessment (SOFA) scores; higher

Table 1
Baseline characteristics of demographics, clinical and laboratory findings in thymosin α1 group and non-thymosin α1 group.

	Total (n = 334)	Non-thymosin α1 (n = 232)	Thymosin α1 (n = 102)	*P*-value
Demographics, clinical characteristics				
Age(year) median (IQR)	57.0 (45.0–67.0)	55.0 (40.0–66.2)	64.0 (56.0–69.0)	< 0.001
Gender (Female) N (%)	139 (41.6%)	105 (45.3%)	34 (33.3%)	0.042
Clinical classifications, N (%)				< 0.001
Critical type	103 (30.8%)	48 (20.7%)	55 (53.9%)	
Severe type	231 (69.2%)	184 (79.3%)	47 (46.1%)	
Comorbidity, N (%)	158 (47.3%)	100 (43.1%)	58 (56.9%)	0.02
Hypertension, N (%)	100 (29.9%)	67 (28.9%)	33 (32.4%)	0.52
Coronary heart disease	31 (9.3%)	21 (9.1%)	10 (9.8%)	0.83
Chronic kidney disease, N (%)	5 (1.5%)	1 (0.4%)	4 (3.9%)	0.032
Diabetes, N (%)	38 (11.4%)	20 (8.6%)	18 (17.6%)	0.017
Chronic obstructive lung disease, N (%)	10 (3.0%)	7 (3.0%)	3 (2.9%)	0.97
Stroke, N (%)	16 (4.8%)	10 (4.3%)	6 (5.9%)	0.54
Carcinoma, N (%)	10 (3.0%)	5 (2.2%)	5 (4.9%)	0.18
Other, N (%)	62 (18.6%)	39 (16.8%)	23 (22.5%)	0.21
Temperature (°C), median (IQR)	37.0 (36.5–37.8)	36.9 (36.5–37.8)	37.0 (36.6–38.0)	0.50
Pulse (beats per min), median (IQR)	88.0 (80.0–97.0)	88.0 (80.0–98.0)	85.0 (80.0–93.8)	0.38
Systolic blood pressure(mmHg), median (IQR)	127.5 (117.0–138.0)	127.0 (117.0–138.0)	129.0 (115.5–138.0)	0.95
Diastolic blood pressure(mmHg), median (IQR)	78.0 (70.0–85.0)	78.5 (70.0–85.8)	78.0 (70.0–83.8)	0.33
Respiratory rate (breaths per min), median (IQR)	20.0 (19.0–22.2)	20.0 (19.0–22.0)	21.0 (20.0–23.0)	0.039
Laboratory findings, median (IQR)				
WBC ($\times 10^9$/L)	5.8 (4.2–8.2)	5.3 (4.1–7.5)	6.5 (4.8–9.1)	0.03
NEU ($\times 10^9$/L)	3.9 (2.6–6.5)	3.6 (2.5–5.8)	4.8 (3.4–8.1)	< 0.001
MON ($\times 10^9$/L)	0.4 (0.3–0.6)	0.4 (0.3–0.6)	0.4 (0.3–0.6)	0.24
LYM ($\times 10^9$/L)	1.0 (0.6–1.4)	1.0 (0.6–1.5)	0.8 (0.6–1.2)	0.005
Critical type	0.7 (0.5–1.0)	0.7 (0.5–1.0)	0.7 (0.5–0.9)	0.98
Severe type	1.1 (0.8–1.6)	1.1 (0.8–1.6)	1.0 (0.6–1.2)	0.26
NLR	3.9 (2.0–9.4)	3.4 (1.8–8.2)	5.5 (3.4–11.7)	0.094
PLT ($\times 10^9$/L)	176.0 (144.0–232.0)	181.0 (145.5–235.5)	171.5 (133.2–219.2)	0.27
HGB (g/L)	129.0 (117.0–141.0)	129.0 (117.0–143.0)	127.5 (117.0–136.5)	0.14
PCT (ng/ml)	0.1 (0.0–0.1)	0.1 (0.0–0.1)	0.1 (0.1–0.2)	0.048
CRP (mg/L)	25.2 (8.8–63.0)	19.5 (7.0–48.2)	37.0 (16.5–85.1)	0.87
IL-6 (pg/ml)	19.1 (7.8–42.4)	16.7 (7.8–40.1)	21.0 (8.3–48.1)	0.11
LACT (mmol/L)	1.6 (1.2–2.1)	1.5 (1.1–1.9)	1.8 (1.2–2.5)	0.006
AST (U/L)	28.1 (21.1–41.5)	28.0 (21.0–41.4)	30.0 (21.9–41.6)	0.15
ALT (U/L)	24.0 (16.1–37.8)	24.0 (16.0–36.9)	26.5 (18.0–39.8)	0.92
TBIL (μmol/L)	11.3 (7.9–15.5)	11.2 (7.4–15.0)	11.6 (8.9–17.4)	0.031
DBIL (μmol/L)	3.7 (2.4–6.0)	3.3 (2.1–5.3)	4.4 (2.9–6.9)	0.029
FIB (g/L)	4.1 (3.4–4.8)	4.1 (3.3–4.7)	4.3 (3.5–5.0)	0.21
CR (μmol/L)	65.0 (52.6–80.8)	63.1 (51.0–78.6)	68.5 (55.2–87.5)	0.028
CK (U/L)	13.1 (0.8–105.5)	23.3 (0.8–127.5)	1.9 (0.9–54.7)	0.014
PaO_2/FiO_2	236.8 (165.0–283.6)	243.0 (190.8–282.7)	205.0 (150.0–282.2)	0.11
Critical type	195.4 (131.5–287.0)	189.8 (96.0–297.2)	195.9 (144.9–285.2)	0.42
Severe type	245.0 (203.3–279.6)	247.3 (214.0–280.8)	225.3 (172.5–270.5)	0.071
APACHE II	6.0 (4.0–9.0)	5.0 (3.0–8.0)	7.0 (5.0–9.0)	0.003
SOFA	2.0 (2.0–3.0)	2.0 (1.0–3.0)	3.0 (2.0–4.0)	< 0.001
Critical type	3.0 (2.0–4.0)	4.5 (3.0–12.8)	4.0 (3.0–5.0)	0.011
Severe type	2.0 (1.0–3.0)	2.0 (1.0–2.0)	2.0 (2.0–3.0)	0.14

IQR, inter-quartile range; APACHE II: Acute Physiology and Chronic Health Evaluation II; SOFA: sequential organ failure assessment; ALT: alanine aminotransferase; AST: aspartate aminotransferase; WBC: white blood cell; NEU: neutrophil; MON: monocytes; LYM: lymphocyte; PLT: platelet; HGB: hemoglobin; PCT: procalcitonin; CRP: C-reactive protein; IL-6: interleutin-6; LACT: lactic acid; TBIL: total bilirubin; DBIL: direct bilirubin; FIB: fibrinogen; CR: creatine; CK: creatine kinase; NLR: neutrophil lymphocyte ratio.

levels of interleukin 6(IL-6), lactate, total bilirubin, and creatinine; and lower lymphocyte counts (all $P < 0.05$, Table 1).

3.2. Primary and secondary outcomes in the overall cohort

Analysis of primary and secondary outcomes in all patients showed that 42 patients (12.6%) died at 28 days and 55 patients (16.5%) died at 60 days; in the thymosin α1 group, 8 patients died within 28 days, and 20 patients died within 60 days. In the non-thymosin α1 group, 34 patients died within 28 days, and 35 patients died within 60 days. There was a significant difference in 28-day mortality between the thymosin α1 group and the non-thymosin α1 group in the adjusted model ($P = 0.016$, Table 2), while no significant differences in 60-day mortality ($P = 1.000$, Table 2) or survival time ($P = 0.81$, supplementary Fig. 1) were found between the two groups.

Analysis of secondary outcomes in all cases revealed that the median hospital length of stay was 20.0 days (IQR 14.0–28.0), and the total course of disease was 27.0 days (IQR 19.0–36.0). Compared with the non-thymosin α1 group, the in-hospital stay and total course of disease of patients were longer in the thymosin α1 group after adjusting for confounders ($P = 0.024$, $P = 0.192$, respectively, Table 2).

3.3. Primary and secondary outcomes in clinical classification subgroups

Subgroup analysis was carried out according to clinical classifications (Table 3). The results showed that, in the adjusted model, treatment with thymosin α1 significantly decreased 28-day mortality ($P = 0.03$, Table 3) but had no marked effects on 60-day mortality ($P = 1.00$, Table 3) in critical type. The hospital length of stay and total course of disease of patients were longer in the thymosin α1 group after adjusting for confounding factors ($P = 0.02$, $P = 0.001$, respectively, Table 3). Moreover, thymosin α1 obviously prolonged survival time in the critical type patients according to log-rank test ($P < 0.0001$ and $P = 0.0006$, respectively, supplementary Figs. 2 and 3). However, in

Table 2
Effects of thymosin α1 treatment on primary and secondary outcomes in overall cohort.

	Total (N = 334)	Non-thymosin α1 (N = 232)	Thymosin α1(N = 102)	*P* value[#]	*P* value[*]
Primary outcomes, N (%)					
28-day mortality	42(12.6%)	34(14.7%)	8(7.8%)	0.084	0.016
60-day mortality	55(16.5%)	35(15.1%)	20 (19.6%)	0.305	1.000
Secondary outcomes, median (IQR)					
In-hospital days	20.0(14.0–28.0)	17.0(13.0–23.0)	28.0(18.2–37.0)	< 0.001	0.024
Total course of disease [a]	27.0(19.0–36.0)	24.0(17.8–34.0)	34.5(27.0–44.0)	< 0.001	0.192

IQR, inter-quartile range; [a] Total course of disease:time from illness onset to death or discharge, days;

[#] Non-adjusted model adjusted for: none.

[*] Adjust model adjusted for: age, gender, comorbidity, PaO_2/FiO_2, lactic acid, procalcitonin, respiratory rate, white blood cell, neutrophil, lymphocyte, total bilirubin, creatine, creatine kinase, SOFA, APACHE II, ulinastatin, Intravenous Immunoglobulin(IVIG), and glucocorticoid.

the severe type patients, there were no differences in either primary or secondary outcomes in the non-thymosin α1 group (all $P > 0.05$, Table 3). The log-rank test showed no marked differences in the 28-day and 60-day survival rates in the severe type patients ($P = 0.091$ and $P = 0.39$, respectively, supplementary Figs. 2 and 3).

3.4. Stratification analysis in clinical classification subgroups

To determine stratification parameters that affected 28-day mortality in clinical classification subgroups, Cox proportional hazards model analysis was performed with age, gender, comorbidities, respiratory rate, white blood cell count, neutrophil, lymphocyte, creatinine, PaO_2/FiO_2, APACHE II score, SOFA score, clinical classification of COVID-19, and interventional measures including ulinastatin, intravenous immunoglobulin (IVIG), and glucocorticoid. It was found that treatment with thymosin α1 significantly decreased 28-day mortality (HR, 0.24; 95% CI 0.08–0.79; $P = 0.018$, supplementary Table 1). The other risk factors associated with 28-day mortality included comorbidities, white blood cells, neutrophils, platelets, SOFA score, and glucocorticoids (HR, 5.62, 0.64, 1.88, 0.99, 1.22, 0.30, respectively, all $P < 0.05$ supplementary Table 1). There were no differences in ΔC-reactive protein, Δlymphocytes, $\Delta PaO_2/FiO_2$, or ΔSOFA, but there were significant differences in ΔIL-6 and Δcreatine in the overall cohort ($P = 0.031$ and $P = 0.013$, respectively, supplementary Table 2).

Based on clinical and The Cox regression results, we performed further analyses with age, comorbidity, white blood cells, neutrophils, platelets, lymphocytes, PaO_2/FiO_2, SOFA, APACHE II, and glucocorticoids. The results showed that thymosin α1 administration was closely associated with decreased 28-day mortality only in the critical patients when age was > 64, white blood cell count was > 6.8×10^9/L, neutrophil count was > 5.3×10^9/L, lymphocyte count was < 0.73×10^9/L, PaO_2/FiO_2 was < 196, SOFA score was > 3, and APACHE II score was > 7, together with comorbidities and glucocorticoid therapy (Table 4).

3.5. Efficacy of thymosin α1 on primary outcomes in clinical classification subgroups

To further confirm the efficacy of thymosin α1 for the primary outcomes of COVID-19 patients, different clinical classifications of thymosin α1 (1.6 mg qd or q12 h for > 5 days) were compared in the present study. In the critical type patients, treatment with thymosin α1 significantly reduced 28-day mortality (HR, 0.11, 95% CI [0.02–0.63], $P = 0.013$, Table 5), improved PaO_2/FiO_2 ($P = 0.036$, supplementary Table 3), and prolonged the hospital length of stay (HR, 9.48, 95% CI [4.71, 14.25], $P < 0.001$, Table 5) as well as the total course of disease (HR, 9.82, 95% CI [4.73–14.90], $P < 0.001$, Table 5). However, in the severe type patients, there were no differences in primary outcomes between the thymosin α1 group and the non-thymosin α1 group (all $P > 0.05$, Table 5). Interestingly, thymosin α1 significantly prolonged the hospital length of stay (HR, 8.84, 95% CI [5.283, 12.40], $P < 0.001$) and the total course of disease (HR, 6.35, 95% CI [2.30, 10.41], $P = 0.002$) in the severe type group (Table 5). No obvious improvements in ΔC-reactive protein, ΔIL-6, Δlymphocytes, Δcreatine, $\Delta PaO_2/FiO_2$, or ΔSOFA were observed following thymosin α1 treatment in the severe type and critical type (all $P > 0.05$, supplementary Table 3).

4. Discussion

In the current multicenter retrospective cohort study, it was revealed that thymosin α1 administration could significantly decrease 28-day mortality among critical type COVID-19 patients, especially those aged over 64 years, with white blood cell counts > 6.8×10^9/L, with neutrophil counts > 5.3×10^9/L, with lymphocyte counts < 0.73×10^9/L, with PaO_2/FiO_2 < 196, with SOFA scores > 3, and with APACHE II scores > 7. Moreover, thymosin α1 obviously attenuated acute lung injury, as evidenced by the improvement in PaO_2/FiO_2 in the thymosin α1 treatment group. Our results provide clinical information concerning target population selection for thymosin α1 therapy for SARS-CoV-2 infection.

SARS-Cov-2 belongs to the coronavirus family, and SARS-CoV-2 infection exhibits similar manifestations and pathophysiological

Table 3
Effects of thymosin α1 on primary and secondary outcomes in subgroups of critical and severe type.

	Critical type			Severe type		
Variables	Non-thymosin α1(n = 48)	Thymosin α1 (n = 55)	*P* value	Non-thymosin α1 (n = 184)	Thymosin α1 (n = 47)	*P* value
Primary outcomes, N (%)						
28-day mortality	29 (60.4%)	7 (12.7%)	0.03	5 (2.7%)	1 (2.1%)	0.99
60-day mortality	30 (62.5%)	19 (34.5%)	1.00	5 (2.7%)	1 (2.1%)	0.99
Secondary outcomes, median (IQR)						
In-hospital days	16.0(10.0–20.2)	28.0(17.0–36.0)	0.02	17.0(13.0–24.0)	28.0(20.5–37.5)	0.13
Total course of disease [a]	25.5(17.8–35.2)	35.0(27.5–44.0)	0.001	23.0(17.8–33.2)	33.0(27.0–43.5)	0.38

IQR, inter-quartile range; [a] Total course of disease:time from illness onset to death or discharge, days

P adjusted for: age, gender, comorbidity, PaO2/FiO2, SOFA, APACHE II, ulinastatin, IVIG, and glucocorticoid.

Table 4
Stratification analysis of thymosin α1 on 28-day mortality in subgroups of critical and severe type.

Variables	Total (N)	Total OR (95% CI) *P* value	Critical type OR (95%CI) *P* value	Severe type OR (95%CI) *P* value
Age (years)				
< 50	109	0.0 (0.0, Inf) 0.995	N/A	0.0 (0.0, Inf) 0.997
51–63	104	2.0 (0.5, 8.5) 0.349	0.7 (0.1, 4.8) 0.679	1.8 (0.2, 20.9) 0.646
> 64	121	0.1 (0.0, 0.4) < 0.001	0.1 (0.0, 0.2) < 0.001	0.0 (0.0, Inf) 0.996
Comorbidity				
No	176	0.8 (0.2, 3.0) 0.748	0.1 (0.0, 0.4) 0.002	2.6 (0.2, 30.3) 0.437
Yes	158	0.3 (0.1, 0.9) 0.028	0.1 (0.0, 0.4) < 0.001	0.0 (0.0, Inf) 0.996
WBC ($\times 10^9$/L)				
< 4.74	101	0.0 (0.0, Inf) 0.996	0.0 (0.0, Inf) 0.997	1.0 (0.0, Inf) 1.000
4.74–6.8	99	0.0 (0.0, Inf) 0.995	0.0 (0.0, Inf) 0.999	0.0 (0.0, Inf) 0.995
> 6.8	102	0.5 (0.2, 1.3) 0.149	0.1 (0.0, 0.5) 0.003	3.8 (0.2, 83.6) 0.397
NEU ($\times 10^9$/L)				
< 3.0	101	0.0 (0.0, Inf) 0.994	N/A	0.0 (0.0, Inf) 0.999
3.0–5.3	101	0.0 (0.0, Inf) 0.999	0.0 (0.0, Inf) 1.000	1.0 (0.0, Inf) 1.000
> 5.3	101	0.4 (0.1, 1.0) 0.058	0.1 (0.0, 0.5) 0.002	0.7 (0.1, 7.4) 0.784
LYM ($\times 10^9$/L)				
< 0.73	101	0.2 (0.0, 0.6) 0.008	0.0 (0.0, 0.2) < 0.001	0.7 (0.1, 7.0) 0.748
0.73–1.2	102	1.0 (0.2, 4.6) 0.971	0.4 (0.1, 2.1) 0.264	1.0 (0.0, Inf) 1.000
> 1.2	102	0.9 (0.1, 9.5) 0.900	0.5 (0.0, 11.6) 0.658	0.0 (0.0, Inf) 0.999
PLT ($\times 10^9$/L)				
< 152	99	0.03 (0.00, 0.60) 0.022	0.0 (0.0, Inf) 0.999	inf. (0.0, Inf) 0.999
152–206	98	0.67 (0.09, 4.91) 0.689	0.4(0.01,16.12) 0.645	2.5 (0.1, 71.7) 0.603
> 206	104	0.3 (0.01, 6.16) 0.423	0.1 (0.0, 8.3) 0.336	0.0(0.0, Inf) 0.999
PaO_2/FiO_2				
< 196	73	0.1 (0.0, 0.3) < 0.001	0.0 (0.0, 0.1) < 0.001	0.4 (0.0, 5.2) 0.506
196–263	73	2.4 (0.3, 18.6) 0.417	2.3 (0.1, 70.3) 0.637	0.0 (0.0, Inf) 0.998
> 264	74	1.1 (0.2, 7.9) 0.889	0.3 (0.0, 3.4) 0.339	1.0 (0.0, Inf) 1.000
SOFA score				
0–1	76	5.0 (0.2, 131.1) 0.338	N/A	0.0 (0.0, Inf) 0.998
2–2	113	0.0 (0.0, Inf) 0.998	1.0 (0.0, Inf) 1.000	0.0 (0.0, Inf) 0.998
> 3	130	0.2 (0.1, 0.6) 0.002	0.1 (0.0, 0.2) < 0.001	0.9 (0.1, 9.8) 0.916
APACHE II				
0–3	73	1.0 (0.0, Inf) 1.000	N/A	1.0 (0.0, Inf) 1.000
4–6	99	0.0 (0.0, Inf) 0.998	1.0 (0.0, Inf) 1.000	0.0 (0.0, Inf) 0.999
> 7	138	0.3 (0.1, 0.7) 0.008	0.1 (0.0, 0.2) < 0.001	0.6 (0.1, 6.0) 0.680
Glucocorticoid				
No	174	0.9 (0.3, 2.7) 0.836	0.2 (0.1, 1.1) 0.062	1.4 (0.1, 15.3) 0.789
Yes	160	0.1 (0.0, 0.6) 0.009	0.0 (0.0, 0.3) < 0.001	0.0 (0.0, Inf) 0.998

WBC: white blood cell; NEU: neutrophil; LYM: lymphocyte; PLT: platelet; SOFA: sequential organ failure assessment; APACHE II: Acute Physiology and Chronic Health Evaluation II.

Adjusted for: age, gender, APACHEII, SOFA, comorbidity, and glucocorticoid.

Table 5
Efficacy of thymosin α1 on primary and secondary outcomes in subgroups of critical and severe type.

Variables	Critical type (N = 103) HR/OR (95%CI) *P* value	Severe type (N = 231) HR/OR (95%CI) *P* value
Primary outcomes, N (%)		
28-day mortality*	0.11 (0.02, 0.63) 0.013	0.55 (0.02, 15.11) 0.725
60-day mortality*	0.53 (0.16, 1.75) 0.30	0.55 (0.02, 15.11) 0.725
Secondary outcomes, median (IQR)		
In-hospital days#	9.48 (4.71, 14.25) < 0.001	8.84 (5.283, 12.40) < 0.001
Total course of disease# [a]	9.82 (4.73, 14.90) < 0.001	6.35 (2.30, 10.41) 0.002

IQR, inter-quartile range; [a] Total course of disease:time from illness onset to death or discharge, days

* Cox model, HR adjusted for: age, gender, comorbidity, white blood cell, neutrophil, lymphocyte, platelet, SOFA, IVIG, ulinastatin, and glucocorticoid.

\# Logistic regression model, OR adjusted for: comorbidity, white blood cell, neutrophil, platelet, SOFA, and glucocorticoid.

processes as other types of coronavirus pneumonia. Although the pathologies of SARS and Middle East respiratory syndrome (MERS) are not yet fully understood, viral invasion and host response appear to be involved in coronavirus infection. When encountered with the virus, the host can trigger immune activation against the virus. Due to the defence of the immune system, over half of patients with SARS-CoV-2 infection manifest mild or no symptoms [14]. However, in critical type cases, patients may experience lymphopenia and pneumonia with high levels of proinflammatory cytokines. The manifestation might be attributed to an out-of-control immune response, which further results in pulmonary tissue damage and even respiratory failure [6]. Thymosin α1 administration has been shown to be efficacious for SARS patients in controlling the development of the disease [15]. Herein, our results showed that treatment with thymosin α1 could decrease 28-day mortality in critical type COVID-19 patients, suggesting that thymosin α1 might improve host immune dysfunction and the poor prognosis of critical type patients.

Several studies have investigated the response of the immune system in COVID-19 patients. Most of these studies have shown that critical COVID-19 patients developed uncontrolled inflammatory activation, resulting in an increase in neutrophils and a decrease in the total number of lymphocytes, which are more significant in critical cases [16]. Of note, lymphocytes play an essential role in antiviral processes by balancing the fight against pathogens and risk, and decreased lymphocytes are related to poor prognosis in many diseases [17,18]. CyTOF and microfluidic qPCR revealed that severe COVID-19 patients showed a decreased T-cell proportion, and T-cell activation as well as differentiation-related genes were downregulated [19]. In this study, our findings confirmed that critical type COVID-19 patients with lower lymphocyte counts could obtain a significant benefit from thymosin α1 therapy.

It is well known that thymosin α1 exerts great biological influence in regulating the function of the immune system in many diseases as an

immune modulator. For instance, thymosin α1 increased the number of activated helper T cells (Th1) and promoted a shift towards the Th1 subset by enhancing T cell maturation and inhibiting T-cell apoptosis [10,11]. Thymosin α1 supplement significantly reduce mortality of severe COVID-19 patients by restoration of lymphocytopenia and reversion of exhausted T cells [20]. Thymosin α1 could activate Toll-like receptor (TLR), leading to stimulation of the nuclear factor kappa B (NF-κB) and p38 mitogen-activated protein kinase (MAPK) pathways, both of which play critical roles in cell maturation [21,22]. SARS-CoV-2 infection not only resulted in decreased lymphocyte counts but also T-cell exhaustion, which manifested as reduced production of effector cytokines, such as IL-2 [23]. It was previously reported that thymosin α1 could help T cells perform their function by activating interferon regulatory factor 7 (IRF7) and upregulating the interferon-γ-dependent effector pathway [24].

Thymosin α1 plays a key role not only in enhancing T cell number and activation but also in favouring antigen presentation. It has been documented that thymosin α1 can augment the expression of major histocompatibility complex (MHC) class I and MHC class II dendritic cells (DCs), which are important for antigen presentation [25]. Activated Th1 cells are important in confronting viral infections and lead to the differentiation of specific B cells [9]. These synergistic effects of T cells, DCs, and B cells enhance the viral clearance of the host and improve organ function in the context of critical illness. As autopsy findings have shown, the lungs were the most seriously damaged among all organs [26]. Our data showed that thymosin α1 markedly improved pulmonary function, as evidenced by elevated PaO_2/FiO_2 in critical type group. Nevertheless, further study should be performed to investigate the key link of the effects of thymosin α1 in critical COVID-19 patients, and the regulatory mechanisms underlying the effects on T cells and DCs after treatment with thymosin α1 should be clarified.

Hyperinflammation and cytokine storms occur in critical patients and contribute to the development of organ dysfunction [27]. A previous study found that thymosin α1 exerted a dual mechanism during inflammation [9,28]. In addition to its impact on enhancing lymphocyte activation, thymosin α1 is able to prevent a proinflammatory cytokine storm by increasing regulatory T cells [12,29]. Moreover, thymosin α1 has the ability to activate DCs through TLR9 signaling. Since TLR9 signaling activates the immunosuppressive pathway via indoleamine 2,3-dioxygenase (IDO), Romani *et al.* [29] noticed that thymosin α1 could help facilitate a balanced control of inflammation and tolerance by targeting IDO-competent DCs.

To our knowledge, this is the first report stating that treatment with thymosin α1 can significantly improve the 28-day survival rate in critical type COVID-19 patients. However, there was no difference in 60-day mortality between the thymosin α1- and non-thymosin α1 treated groups, and the potential mechanism remains unknown. One of the possible explanations might be the short duration of thymosin α1 treatment. Therefore, the long-term effect of thymosin α1 should be further evaluated. In addition, thymosin α1 administration failed to reverse late-stage death, which may be due to the serious condition of the patients. Among the 55 deaths that occurred during this observation, 13 occurred after 28 days, and most of the patients had a baseline APACHE II score over 10. Since most deaths occurred within 28 days, early use of thymosin α1 could decrease 28-day mortality in critical type COVID-19 patients. Therefore, thymosin α1 therapy might be beneficial for critical type COVID-19 patients.

In conclusion, treatment with thymosin α1 can decrease 28-day mortality and attenuate organ dysfunction in critical type COVID-19 patients, and our findings provide clinical information with regard to target population selection for thymosin α1 therapy in the setting of SARS-CoV-2 infection. Further study is needed to investigate the underlying mechanisms, dosage and duration of thymosin α1 therapy for critical ill patients with COVID-19.

Contributors

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. YY, LZ, SL and WM were responsible for study concept and design. LZ, FY, WM, WC, LZY, XQ, and SZ were responsible for collecting the data. WM, LZ, and JJ were responsible for statistical analysis. YY, LZ, WM and JJ were responsible for drafting the manuscript.

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CRediT authorship contribution statement

Ming Wu: Conceptualization, Methodology, Formal analysis, Writing - original draft, Funding acquisition. **Jing-jing Ji:** Formal analysis, Writing - original draft, Writing - original draft. **Li Zhong:** . **Zi-yun Shao:** Data curation. **Qi-feng Xie:** Data curation, Funding acquisition. **Zhe-ying Liu:** Data curation. **Cong-lin Wang:** Data curation. **Lei Su:** Conceptualization, Methodology. **Yong-wen Feng:** Data curation, Funding acquisition, Validation, Funding acquisition. **Zhi-feng Liu:** Conceptualization, Methodology, Data curation, Funding acquisition. **Yong-ming Yao:** Conceptualization, Methodology, Funding acquisition, Supervision, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2020.106873.

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