Original Article

Beneficial aspects of high dose intravenous vitamin C on patients with COVID-19 pneumonia in severe condition: a retrospective case series study

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Background: Coronavirus disease 2019 (COVID-19) is a global public health event without specific therapeutic agents till now. We aim to determine if high dose intravenous vitamin C (HDIVC) was effective for COVID-19 patients in severe condition.

Methods: COVID-19 patients admitted in Shanghai Public Health Clinical Center from January 22, 2020 to April 11, 2020 were retrospectively scrolled. The enrolled patients were those with confirmed diagnosis of severe or critical COVID-19 pneumonia, who received HDIVC within 24 hours after disease aggravation. Main clinical outcomes obtained from 3–5 days (day 3) and 7–10 days (day 7) after HDIVC were compared to the ones just before (day 0) HDIVC.

Results: Totally, twelve patients were enrolled including six severe [age of mean, 56; interquartile range (IQR), 32–65 years, 3 men] and six critical (age of mean, 63; IQR, 60–82 years, 4 men) patients. The dosage of vitamin C [median (IQR), mg/kg (body weight)/day] were 162.7 (71.1–328.6) for severe and 178.6 (133.3–350.6) for critical patients. By Generalized estimating equation (GEE) model, C-reactive protein (CRP) was found to decrease significantly from day 0 to 3 and 7 (severe: 59.01±37.9, 12.36±22.12, 8.95±20.4 mg/L; critical: 92.5±41.21, 33.9±30.2, 59.56±41.4 mg/L). Lymphocyte and CD4+ T cell counts in severe patients reached to normal level since day 3. Similar improving trends were observed for PaO_2/FiO_2 (severe: 209.3±111.7, 313.4±146, 423.3±140.8; critical: 119.9±52.7, 201.8±86.64, 190.5±51.99) and sequential organ failure assessment score (severe: 2.83±1.72, 1.33±1.63, 0.67±1.03; critical: 6.67±2.34, 4.17±2.32, 3.83±2.56). Better improving effect was observed in severe than critical patients after HDIVC.

Conclusions: HDIVC might be beneficial in aspects of inflammatory response, immune and organ function for aggravation of COVID-19 patients. Further clinical trials are in warrant.

Trial registration: This trial has been retrospectively registered in Chinese Clinical Trail Registry (ChiCTR2000032716) on May 8, 2020. http://www.chictr.org.cn/showproj.aspx?proj=53389.

Keywords: Coronavirus disease 2019 (COVID-19); vitamin C; aggravation; therapy; C-reactive protein (CRP)

Submitted Jul 09, 2020. Accepted for publication Sep 28, 2020.
doi: 10.21037/apm-20-1387
View this article at: http://dx.doi.org/10.21037/apm-20-1387

Introduction

Coronavirus disease 2019 (COVID-19), the infectious illness caused by severe acute respiratory coronavirus 2 (SARS-CoV-2) has become a worldwide heath care crisis since December 2019 (1,2). The severity of COVID-19 is classified into mild, moderate, severe and critical type on the guideline made by National Health and Family Planning Commission of the People's Republic of China (3). The severe patient is mainly characterized with deteriorated respiratory function and rapid progression of radiological lesions, and the critical patient further requires mechanical ventilation (MV), accompanying with shock or multiple organ failure (Table S1). The aged patients and those with pre-existing respiratory, cardiac and diabetic disease appear to be at high risk of rapid development to severe and critical type (4). Various methods of respiratory support are the main treatment for the severe and critical patients, especially in the situation of disease aggravation. The respiratory supports are consisted of high flow oxygen therapy, noninvasive or invasive MV and extracorporeal membrane oxygenation (ECMO). As effective antiviral therapy is still vacant, the mortality of COVID-19 patients who required MV was reported as high as 66% (5). Therefore, it is important to develop an effective rescue therapy for these patients, especially in the situation of disease aggravation.

Vitamin C, one of the key antioxidants of the body, has been highlighted recently in the field of critical care medicine (6). High does intravenous vitamin C (HDIVC), a new developed method of administration, has been proven to exert beneficial effect in various critical illness by animal and clinical study (7,8). The rationale for HDIVC application in the treatment of severe and critical COVID-19 patients, as we deemed, relies on (I) its effective ability for eliminating the burst of reactive oxygenase species (ROS) and the following cytokine storm (9), which are the basis for acute respiratory distress syndrome (ARDS). The recent CITRIS-ALI study (10) showed HDIVC (50 mg/kg actual body weight every 6 hours for 96 hours) reduced 28 day all-cause mortality (29.8% *vs.* 46.3%) in a cohort of septic patients with ARDS. (II) Its immune enhancing property and the potential anti-virus ability (11), which might help for improving lymphopenia, the main characteristic of COVID-19 which was related with severity (12). Also, application of HDIVC in COVID-19 has already got concerns of some scholars (13). Still, promotion of HDIVC needs further evidence.

In this retrospective case series study, we made an observational comparison of outcomes before and after HDIVC in a cohort of severe and critical patients after disease aggravation occurred. The dosage of vitamin C [median (IQR)] applied in severe patients was 162.7 (71.1–328.6) mg/kg (body weight)/day and 178.6 (133.3–350.6) mg/kg/day in critical patients. The main outcomes were mainly divided into inflammatory response, immune and organ dysfunction. To the best of our knowledge, it is the first time to describe the effect of HDIVC on COVID-19 pneumonia. We present the following article according to the STROBE reporting checklist (available at http://dx.doi.org/10.21037/apm-20-1387).

Methods

Study design and participants

This study is a retrospective case series study. It was conducted in accordance with the amended Declaration of Helsinki (as revised in 2013) and was approved by the institutional ethics board of Ruijin Hospital, Shanghai Jiaotong University school of medicine. Oral consents were obtained from each enrolled patient. COVID-19 patients admitted in Shanghai public health clinical center from January 22, 2020 to April 11, 2020 were retrospectively scrolled. The inclusion criteria were: (I) age >18 years; (II) patients who was diagnosed with severe or critical COVID-19 pneumonia. Diagnosis and severity classification were based on the guideline made by National Health and Family Planning Commission of the People's Republic of China (3) (the specific criteria of severity was shown in Table S1). (III) The severe and critical patients

suffered with disease aggravation which was defined as continuous decreasing of PaO_2/FiO_2 or enlargement of lung radiological lesion for 1–3 days. (IV) The enrolled patients should accept HDIVC within 24 hours after disease aggravation. The judgement of aggravation occurrence and the initiation of HDIVC was performed by Shanghai COVID-19 pneumonia expert group; the dosage of vitamin C was determined according to the common opinion of expert group based on the previous clinical study and research work. (V) patients without being pregnant; (VI) patients without malignant tumor.

Data collection

All the data were collected from electronic medical forms by the same trained physician team. The general information including age, gender, body weight and co-existing diseases were collected. The interval from first symptom to admission and initiation of HDIVC were calculated. The dosage, duration and contemporary maximum oxygen support and other medication (including antibiotics, glucocorticoid and low molecular heparin) were collected. The worst value of outcomes on the day before HDIVC (named as day 0), 3–5 (named as day 3) and 7–10 (named as day 7) days after HDIVC were collected. As the intensive monitoring performed immediately after aggravation occurred, all the data of day 0 could be obtained. Most data of days 3 and 7 could also be obtained since the formulated clinical routine test. Outcomes included inflammatory markers [serum level of C-reactive protein (CRP), body temperature, erythrocyte sedimentation rate (ESR)], immune function indicators (counts of lymphocyte and $CD4^+$ T cell), organ function indicators [PaO_2/FiO_2, D-Dimer, platelet counts, sequential organ failure assessment (SOFA) score, serum level of lactate dehydrogenase (LDH), total bilirubin and creatinine].

Statistical analysis

Continuous variables were presented as medians with interquartile ranges (IQRs) and compared using Mann-Whitney U test or reported as mean with standard deviation and compared with *t*-test. Categorical variables were presented as frequency with percentage and compared with Fisher's exact test. Generalized estimating equation (GEE) approach was applied to investigate the effect of HDIVC on inflammatory markers, immune function, and organ function longitudinally over time, adjusted for severity type. All statistical analyses were performed using SAS v. 9.2 (SAS Institute Inc., USA). Two-sided P values of less than 0.05 were deemed to be statistically significant.

Results

Patients characteristics

A total of twelve COVID-19 patients were enrolled, among which six of them were classified into severe type, and other six critical type (Figure S1). As *Table 1* showed, the median age of severe and critical type was 56 years (IQR, 32–65 years) and 63 years (IQR, 60–82 years) respectively. Three of six were men among severe patients and four of six among critical patients. For severe patients, the median interval from first symptom to admission and HDIVC initiation was 3.5 days (IQR, 2–5.5 days) and 8 days (IQR, 7.8–10.3 days), and for critical patients, the interval was 5 days (IQR, 3.8–10.3 days) and 19 days (IQR, 11.25–21.25 days). Between severe and critical patients, no statistically significant differences were found regarding age (P=0.26), body weight (P=0.47) and interval from onset to admission (P=0.23). The interval from disease onset to HDIVC initiation was longer in critical than severe patients with borderline significance (P=0.05). The duration and dosage of HDIVC showed no evidence of difference between two types (P>0.05). The dosage of vitamin C [median (IQR)] applied in severe patients was 162.7 (71.1–328.6) mg/kg (body weight)/day and 178.6 (133.3–350.6) mg/kg/day in critical patients. Among severe patients, four cases were treated with oxygen therapy of nasal cannula with 3 litters/L, one case with high flow oxygen therapy and one case with non-invasive positive pressure ventilation. All critical patients were treated with mechanical ventilation (MV). Among them, three cases also accepted ECMO, two cases received vasoactive drug due to circulatory failure, one case received continuous renal replacement therapy. Other contemporary treatments accompanying with HDIVC after disease aggravation included glucocorticoid, antibiotics, low molecular heparin (*Table 1*). The length of stay was significantly longer in critical [67 (IQR, 39–70) days] than severe patients [17 (IQR, 14–15) days; P=0.02].

HDIVC on inflammatory response

Elevation of serum CRP (*Table 2*) level was observed

Table 1 Characteristics of COVID-19 patients

Characteristic	Severe (n=6)	Critical (n=6)	P value
Age, median (IQR), y	56 [32–65]	63 [60–82]	0.26
Sex (male, n)	3	4	1.00
Weight, median (IQR), Kg	72.5 (66.5–88.75)	70 (62.5–75)	0.47
Co-existing chronic disease			
Hypertension, n	3	3	1.00
Diabetes, n	1	0	1.00
Cardiac heart disease, n	0	0	–
Interval from first symptom, median (IQR), days			
To admission	3.5 (2–5.5)	5 (3.8–10.3)	0.23
To HDIVC initiation	8 (7.75–10.3)	19 (11.25–21.25)	0.05
HDIVC dosage, median (IQR), mg/kg (body weight)/days	162.7 (71.1–328.6)	178.6 (133.3–350.6)	0.67
HDIVC duration, median (IQR), days	5 (1–10.5)	4.5 (1–16.5)	0.62
Maximum oxygen support, n			
Nasal cannula	4	0	0.06
NIPPV	1	0	1.00
High flow	1	0	1.00
Mechanical ventilation	0	6	0.002
ECMO	0	3	0.18
Other contemporary treatments, n			
Glucocorticoid	3	1	0.55
Antibiotic	4	2	0.57
Low molecular heparin	1	2	1.00
Vasoactive drug	0	2	0.46
Renal replacement	0	1	1.00
Length of stay, median (IQR), d	17 [14–15]	67 [39–70]	0.002

HDIVC, high dose intravenous vitamin C; IQR, interquartile range; NIPPV, non-invasive positive pressure ventilation; ECMO, extracorporeal membrane oxygenation.

in both severe (59.01±37.9 mg/L) and critical (92.5± 41.21 mg/L) patients as aggravation occurred, which then decreased continuously (*Figure 1A*) at day 3 (12.36± 22.12 mg/L) and day 7 (8.95±20.4 mg/L) in severe patients. For critical patients, CRP decreased at day 3 (33.9± 30.2 mg/L) and then slightly increased at day 7 (59.56± 41.4 mg/L). The body temperature (*Table 2*) which partly reflects inflammatory response decreased continuously to almost normal level along with time after HDIVC in both cohorts (*Figure 1B*). However, the ESR did not show decreasing trend after HDIVC. GEE models (*Table 3*) further proved the downregulating effect of HDIVC on CRP at day 3 (P<0.0001) and day 7 (P=0.003) days as well as the body temperature at day 3 (P<0.0001) and day 7 (P<0.0001) days in both cohorts. The ameliorating effect of HDIVC on CRP in severe patients was statistically better

Table 2 Trend of indicators for the inflammatory response, immune and organ function before and after HDIVC

Indicators (mean ± SD)	Normal range	Severe			Critical		
		Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
Inflammatory response							
CRP, mg/L	<0.499	59.01±37.9	12.36±22.12*	8.95±20.4*	92.5±41.21	33.9±30.2*	59.56±41.4*
ESR, mm/h	0–20	84.5±31.4	76±36.2	80.8±35.8	96.3±16.7	66.5±38.1	84.8±20.52
Body temperature, ℃	36–37	38.8±1.48	36.8±0.68*	36.7±0.49*	38.8±0.71	37.3±0.91*	37.02±0.72*
Immune function							
Lymphocyte counts, $\times10^9$/L	1.1–3.2	0.88±0.42	1.52±0.64*	2.39±1.85*	0.52±0.31	1.0±0.74*	0.64±0.39*
$CD4^+$ T-cell counts, n/L	410–1,590	350.9±389.1	597.9±212.5*	884.7±368.2*	223.9±131	361.6±165*	263.2±138.2*
Organ function							
PaO_2/FiO_2	0–300	209.3±111.7	313.4±146*	423.3±140.8*	119.9±52.7	201.8±86.64*	190.5±51.99*
Platelet count, $\times10^9$/L	125–350	195±71.97	288.5±93.38	331±97.08	160±106.4	166.3±79.71	158.2±76.17
D-dimer, g/mL	0–0.5	1.17±1.287	0.93±0.35*	0.96±0.72	12.13±6.93	5.27±3.63*	8.34±8.29
TB, mol/L	3.4–20.5	8.817±1.751	7.7±1.529	8.767±1.919	19±3.969	18.28±2.772	16.23±1.283
LDH, U/L	106–211	421.2±161.8	365.3±86.9	287.7±67.2*	547.5±128.1	486.5±140.5	347.8±64.5*
Creatinine, mol/L	53–97	63.53±12.09	46.77±3.386*	52.73±3.152	110.9±43.03	101.6±43.35*	109.1±49.51
SOFA score	0	2.83±1.72	1.33±1.63*	0.67±1.03*	6.67±2.34	4.17±2.32*	3.83±2.56*

Day 0: the day before HDIVC; Day 3: 3–5 days after HDIVC; Day 5: 7–10 days after HDIVC. HDIVC, high dose intravenous vitamin C; SOFA, sequential organ failure assessment; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; LDH, lactic dehydrogenase; TB, total bilirubin. *, P<0.05 compared to day 0.

than the one in critical patients (P=0.0125).

HDIVC on immune function

On aggravation, lymphocytopenia (*Table 2*) occurred in both severe (0.88±0.42, $\times10^9$/L) and critical (0.52±0.31, $\times10^9$/L) patients, and its subgroup $CD4^+$ T-cell (*Table 2*) showed the same trend (severe: 350.9±389.1, number/L; critical: 223.9±131, number/L). At days 3 and 7, the lymphocyte (1.52±0.64, 2.39±1.85, $\times10^9$/L, *Figure 1C*) and $CD4^+$ T-cell (597.9±212.5, 884.7±368.2, number/l, *Figure 1D*) counts renormalized in severe patients. For critical patients, the lymphocytopenia remained after HDIVC, and $CD4^+$ cell counts increased at day 3 with following decreased at day 7. The GEE models (*Table 3*) showed the general increasing trend of lymphocyte and $CD4^+$ T cell counts at days 3 and 7 after HDIVC (all P<0.05). The immune function enhancing profile were more marked in severe group compared to critical group after HDIVC (P=0.0109 for lymphocyte, P=0.0017 for $CD4^+$ T cell).

HDIVC on organ functions

PaO_2/FiO_2 (*Table 2*), representing the respiratory function, decreased after aggravation (209.3±111.7) and returned to normal level at day 3 (313.4±146) and day 7 (423.3±140.8) in severe patients (*Figure 1E*). For critical patients, PaO_2/FiO_2 increased from day 0 (119.9±52.7) to day 3 (201.8±86.64) and day 7 (190.5±51.99). By GEE model (*Table 3*), the HDIVC related improving effect was proved (P=0.0011 for day 3, 0.0007 for day 7). The difference of HDIVC improving effect between severe and critical type was significant (P=0.001). D-Dimer (*Table 2*) representing coagulation function slightly increased in severe group (1.17±1.287, mg/mL) when aggravation occurred but with a greater extent in critical group (12.13±6.93, mg/mL). After HDIVC treatment, D-Dimer in critical patient decreased markedly at day 3 but not at day 7 in both groups. In GEE model (*Table 3*), significant difference was found in decreasing of D-Dimer between day 3 (not day 7) versus day 0. The improving effect of HDIVC on D-Dimer

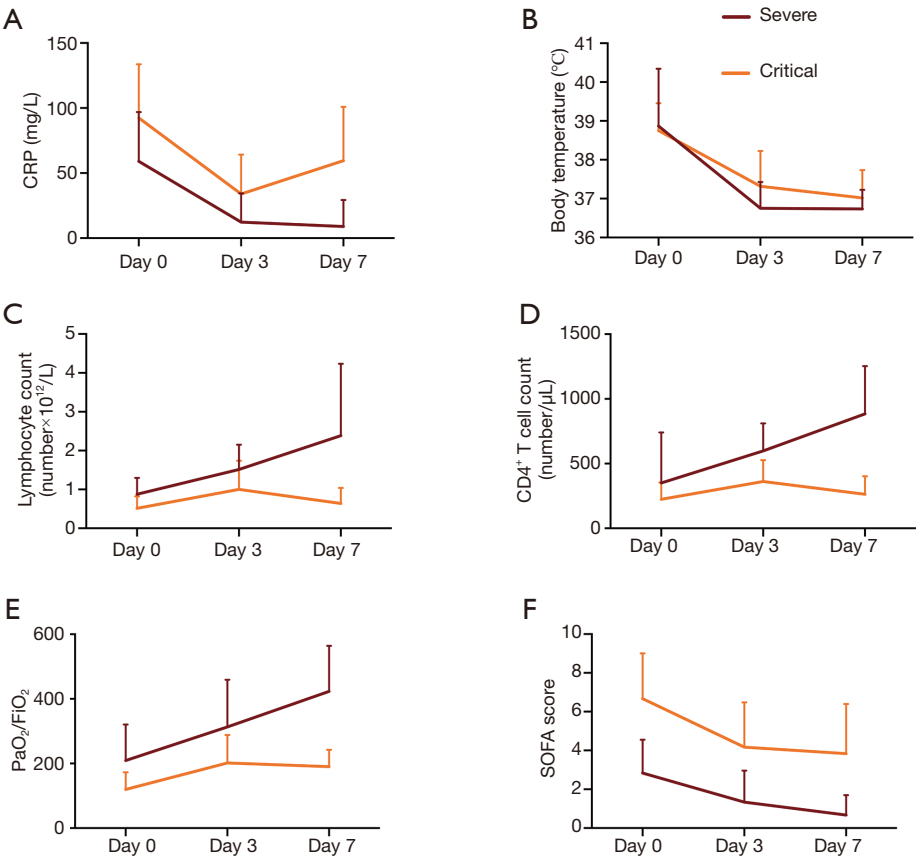

Figure 1 Trend of inflammatory markers, immune and organ function indicators after HDIVC. Note: inflammatory markers include CRP and body temperature, immune function indicators include count of lymphocytes and $CD4^+$ T cell, organ function indicators include PaO_2/FiO_2 and SOFA score. The worse value of variables on the day before HDIVC (day 0), 3–5 (named as day 3) and 7–10 (named as day 7) days were collected. All the variables significantly improved at day 3 and 7 compared to day 7. Dark red short line indicates the COVID-19 patients with severe type. Yellow short line indicates the COVID19 with critical type. CRP, C-reactive protein; SOFA, sequential organ failure assessment; COVID-19, coronavirus disease 2019.

was better in critical than severe group (P<0.0001). PLT (*Table 2*) counts stayed in normal range during the observational period. The liver function indicator LDH (*Table 2*) level increased abnormally as aggravation occurred in severe (421.2±161.8 U/L) and critical (547.5±128.1 U/L) group, and showed decreasing trend in both groups. GEE model showed significant decrease of LDH only occurred at day 7 (P=0.0002) but not day 3 as compared to day 0. The improving effect of HDIVC on LDH was better in critical than severe group (P=0.0122). Another liver function total bilirubin (*Table 2*) was among the normal range after HDIVC. Accordingly, the SOFA score (*Table 2*), contributed mainly by respiratory dysfunction in severe and critical patients, showed the same decreasing trend as PaO_2/FiO_2 did at days 3 and 7 after HDIVC (*Figure 1F*). SOFA score (*Table 3*) improved better in severe group than critical group by HDIVC as GEE model showed (P=0.0004).

Discussion

In this retrospective case series study, we mainly focused on the effect of HIDVC on COVID-19 in three aspects: inflammatory response, immune function and organ function. We showed improvement of CRP, body temperature, lymphocyte counts, $CD4^+$ T cell counts, PaO_2/FiO_2 and SOFA score within ten days after HDIVC in two cohorts of severe and critical patients (Figure S2). We also found the improving effect of HDIVC on the outcomes of severe patients was better than the one of critical patients.

In critical ill patients, serum level of vitamin C is

Table 3 Effect of HDIVC on inflammatory markers, immune function and organ function over time and between severe and critical types

Indicators	Parameter	Level	β	SE	P
CRP	Timepoint	Day 3	−52.6	10.0	<0.0001
		Day 7	−41.5	11.5	0.0003
	Type	Severe	−35.2	14.1	0.01
ESR	Timepoint	Day 3	−18.5	12.6	0.14
		Day 7	−7.7	9.5	0.42
	Group	Severe	−3.1	11.8	0.79
Body temperature	Timepoint	Day 3	−1.8	0.4	<0.0001
		Day 7	−1.9	0.4	<0.0001
	Type	Severe	−0.2	0.3	0.38
Lymphocyte counts	Timepoint	Day 3	0.6	0.2	0.0005
		Day 7	0.8	0.4	0.03
	Type	Severe	0.9	0.3	0.01
$CD4^+$ T cell counts	Timepoint	Day 3	202.2	80.7	0.01
		Day 7	286.5	97.1	0.003
	Type	Severe	334.8	106.7	0.002
PaO_2/FiO_2	Timepoint	Day 3	93.0	28.5	0.001
		Day 7	142.3	42.1	0.0007
	Type	Severe	144.6	43.8	0.0010
Platelet counts	Timepoint	Day 3	49.9	21.5	0.02
		Day 7	67.1	34.4	0.05
	Type	Severe	110.0	39.1	0.005
D-Dimer	Timepoint	Day 3	−3.5	1.3	0.005
		Day 7	−2.0	2.0	0.32
	Type	Severe	−7.6	1.8	<0.0001
LDH	Timepoint	Day 3	−58.4	37.3	0.12
		Day 7	−166.6	44.0	0.0002
	Type	Severe	−102.6	40.9	0.01
TB	Timepoint	Day 3	−0.9167	1.3088	0.48
		Day 7	−1.4083	1.4458	0.33
	Group	Severe	−9.4111	2.7385	0.0006
Creatinine	Timepoint	Day 3	−13.0217	6.3536	0.04
		Day 7	−6.3192	6.7262	0.35
	Type	Severe	−52.8644	41.4763	0.20
SOFA score	Timepoint	Day 3	−2.0	0.5	<0.0001
		Day 7	−2.5	0.6	<0.0001
	Type	Severe	−3.3	0.9	0.0004

Generalized estimating equation (GEE) approach was applied to investigate the effect of HDIVC on inflammatory markers, immune function and organ function longitudinally over time, adjusted for severity type. β>0 means: (I) the value of D3 or D7 is greater the one of D0; (II) the value of severe group is greater than the one of critical group. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LDH, lactic dehydrogenase; TB, total bilirubin SOFA, sequential organ failure assessment.

reported to drop to 10–30 mol/L (normal level of 80–100 mol/L). This might be related to rapid exhaustion by ROS, dilution due to rapid fluid resuscitation, insufficiency supplement and elimination by renal replacement therapy (14). Vitamin C deficiency was reported correlated with the occurrence of multiple organ failure in critically ill patients (15), and requirement for vitamin C increase with diseases severity (16). As genetically loss of the ability to synthesize vitamin C, human must obtain exogenous vitamin C. Intravenous administration of vitamin C showed excellent supplementary effect by quickly increasing the serum level from mol/L to mmol/L (17). Therefore, it is rational to apply HDIVC in the rescue therapy of COVID-19 in severe condition. Recently, treatment with HDIVC for severe acute respiratory distress syndrome (ARDS) from COVID-19 was recommended (18).

The elevation of serum CRP was observed in both severe and critical groups which were consistent with other studies describing the characteristic of COVID-19 patients in severe situation (12,19). The kinetics of CRP is usually used to track and monitor the inflammatory response caused by infection as its short half-life of 19 hours (20). CRP level was shown with rapid reduction by HDIVC (200 mg/kg/day) in a previous before after study in a cohort of sepsis patient (21). Similarly, we showed CRP decreased significantly within ten days after HDIVC by building GEE models. Combined with the trend of body temperature, we deemed that HDIVC might be beneficial for the inflammatory response in COVID-19 patients.

Lymphocytopenia is main characteristic of COVID-19 patients. Recently, the reduction of lymphocyte subset including $CD4^+$, $CD8^+$ and $CD3^+$ T cell was reported to be correlated with COVID-19 severity (22). The underlying mechanism is complex, mainly contributed to the invasion of virus itself. SARS-CoV-2 might causes growth inhibition and apoptosis of hematopoiesis by promotion of autoimmune antibody (23). SARS-CoV-2 also leads the apoptosis of T lymphocyte by affected dendritic cell and production of certain cytokine (24). Consistent with these studies, we showed lymphocyte and its subgroup $CD4^+$ T cell was in subnormal level as aggravation occurred. Lymphocytes, especially T lymphocyte, have been extensively studied in the context of vitamin C biology (25). Both *in vitro* and *in vivo* studies showed vitamin C was essential for the development, maturation and proliferation of functional T-lymphocytes and epigenetic regulation of gene expression is one of the underlying mechanisms (26). In our study, we showed lymphocyte and its subgroup $CD4^+$ T cell returned to normal level in severe patients and increased markedly in critical patients after HDIVC treatment. Our findings partly supported the immune enhancing profile of HDIVC in the treatment of COVID-19.

Multiple organs failure was reported in COVID-19 patients with severe situation (12). The most notable involved organ is the lung. The disease aggravation often started with a rapid development of respiratory failure and decreased PaO_2/FiO_2. Other organ systems secondary to lung include coagulation system, kidney, liver and heart. Decreased platelet counts and elevation of D-D dimer, serum of creatinine, lactic dehydrogenase, glutamic oxaloacetic transaminase, cardiac troponin and creatinine kinase were usually found in severe and critical patients (12). This phenomenon was also partly shown by our study. The organ protection by HDIVC was proved by preclinical and clinical studies in various models of critical condition (27,28). The mechanism relies on the rapid scavenging of ROS and attenuation of ROS related endothelial dysfunction which cause multiple organ failure, especially the ARDS. Other specific mechanisms of HDIVC preventing ARDS include the regulatory of NETosis (29), reducing inflammation via attenuation of NF-κB activation (30), enhancing lung epithelial barrier function by promoting epigenetic and transcriptional expression of protein channels at the alveolar capillary membrane which help regulate alveolar fluid clearance (31). In our study, PaO_2/FiO_2 were significantly improved after HDIVC in both severe and critical patients. It is noted that the superior respiratory support, such as MV or ECMO, had been already applied before the initiation of HDIVC, therefore the increase of PaO_2/FiO_2 was probably contributed to usage of HDIVC but not the enhancement of respiratory support. SOFA score also showed decreasing trend after HDIVC, and this result are consistent with Fowler *et al.*'s early findings (21). However, in their latest prospective study named as CITRIS-ALI trial (10), Fowler *et al.*'s showed opposite result with much larger sample size. The dosage of vitamin C in their study was 50 mg/kg (body weight) per 6 hours which are similar as ours [162.7 (71.1–328.6) mg/kg/day for severe and 178.6 (133.3–350.6) mg/kg/day for critical patients]. The inconsistence between our study and CITRIS-ALI trial is mainly due to the trial design, and the varies etiologies of enrolled patients and their higher SOFS score (9.8–10.3).

Besides, GEE model showed HDIVC exert better improving effect on the outcomes of severe than the one

of critical patients. This might be explained as: (I) the difference of severity between two cohorts, and the severe patients might get better prognosis than critical patients as they received the almost same dosage of vitamin C (*Table 1*); (II) the interval from admission to HDIVC initiation of severe patients [8 (IQR, 7.8–10.3) days] is shorter than the one of critical patients [19 (IQR, 11.25–21.25) days]. Although without statistically difference (P=0.05), it implies that the earlier application of HDIVC could gain more benefits.

It is reported that high dose, long term, oral application of vitamin C was related with the formation of renal oxalate stone (32) which might cause potential harm to renal function. Therefore, the safety profile of vitamin C should be concerned. The latest case serious study including 157 patients showed no renal stone was reported after temporary, intravenous, high dose application of vitamin C (33). In our study, no patient was observed to suffer any identifiable adverse event related with HDIVC.

Limitation: (I) relative fewer patients accepted HDIVC rescue therapy restrained the study design as the before and after treatment study and small sample size. No controlled group was applied. These defects limit the power of study; (II) the dosage and duration varied relative widely depending on clinical situation and clinician experience. Based on the experiences obtained from these cases and our previous work, we promoted a HDIVC protocol (Figure S3) and its related prospective controlled clinical trial (ChiCTR2000032400) are being conducted now; (III) the interfering effect of concomitant therapies such as glucocorticoid, antibiotic, low molecular heparin could not be excluded. Therefore, HDIVC might be regarded as compassionate similar to Remdesivir reported recently (34); (IV) the regression to the mean is an unavoidable phenomenon, which might overstate the therapeutic effect of HDIVC.

Conclusions

In the preliminary retrospective study, HDIVC might be regarded as an important rescue therapy in aggravation of severe and critical COVID-19 patients according to the observational improvement of inflammatory response, immune function and organ function. The limited sample size and study design preclude a definitive statement about the potential effectiveness of HDIVC, and these observations require evaluation in clinical trials.

Acknowledgments

We thank all the staff of Shanghai public health clinical center for their great effort on the treatment of COVID-19 pneumonia patients.

Funding: The second batch of emergency key scientific and technological project of Shanghai Municipal Committee of Science and Technology (20411950300/20411950301). The Clinical Research Project of Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (2018CR004) to Enqiang Mao. National Natural Science Foundation of China (81870311) to Jun Huang.

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at http://dx.doi.org/10.21037/apm-20-1387

Data Sharing Statement: Available at http://dx.doi.org/10.21037/apm-20-1387

Peer Review File: Available at http://dx.doi.org/10.21037/apm-20-1387

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apm-20-1387). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. It was conducted in accordance with the amended Declaration of Helsinki (as revised in 2013) and was approved by the institutional ethics board of Ruijin Hospital, Shanghai Jiaotong University school of medicine. Oral consents were obtained from each enrolled patient.

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Cite this article as: Zhao B, Ling Y, Li J, Peng Y, Huang J, Wang Y, Qu H, Gao Y, Li Y, Hu B, Lu S, Lu H, Zhang W, Mao E. Beneficial aspects of high dose intravenous vitamin C on patients with COVID-19 pneumonia in severe condition: a retrospective case series study. Ann Palliat Med 2021;10(2):1599-1609. doi: 10.21037/apm-20-1387

Supplementary

Table S1 Classification of COVID-19 severity

Severity	Severity description
Mild	The clinical symptoms were mild, and no pneumonia manifested on imaging examination
Moderate	With fever, respiratory tract and other symptoms, imaging examination showed pneumonia
Severe	Meet any of the followings: 1. Shortness of breath, respiratory rate ≥30 times/min; 2. In resting state, arterial oxygen saturation (SaO_2) ≤93%; arterial partial pressure of oxygen, PaO_2)/fraction of inspired oxygen (FiO_2) ≤300 mmHg (1 mmHg =0.133 kPa). At high altitudes (above 1,000 m), PaO_2/FiO_2 should be corrected according to the following formula: PaO_2/FiO_2 × [Atmospheric Pressure (mmHg)/760]; 3. Pulmonary imaging showing lesions progressed significantly within 24 to 48 hours, and those with more than 50% of the lesions were managed as severe
Critical	Meet any of the followings: 1. Require mechanical ventilation; 2. Shock occurs; 3. Combination with other organ failure requiring ICU monitoring and treatment

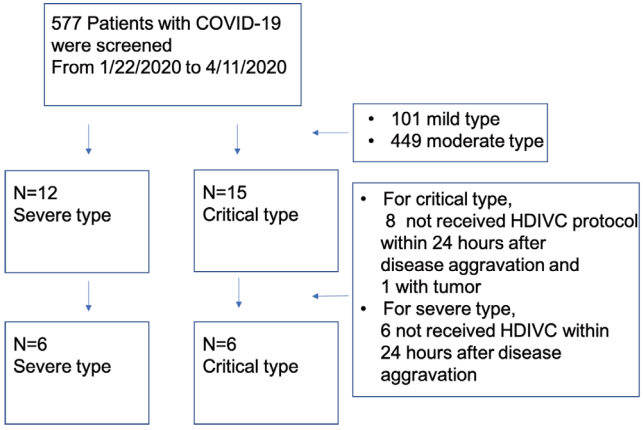

Figure S1 Flowchart of the study. HDIVC, high dose intravenous vitamin C; COVID-19, coronavirus disease 2019.

http://dx.doi.org/10.21037/apm-20-1387

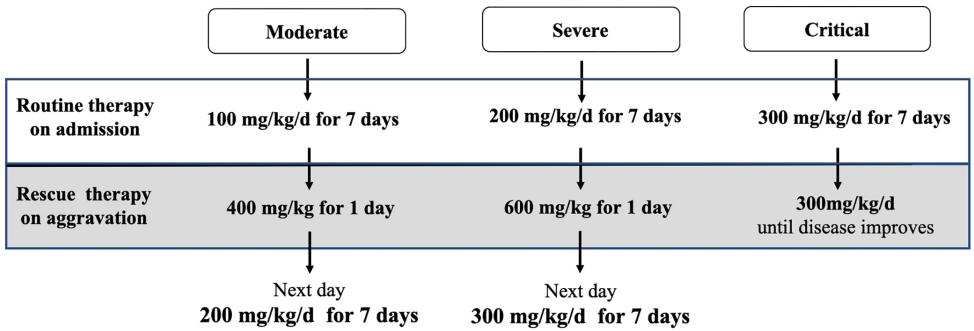

Figure S2 The potential beneficial effect of HDIVC on COVID-19 pneumonia. CRP, C-reactive protein; SOFA, sequential organ failure assessment; HDIVC, high dose intravenous vitamin C; COVID-19, coronavirus disease 2019.

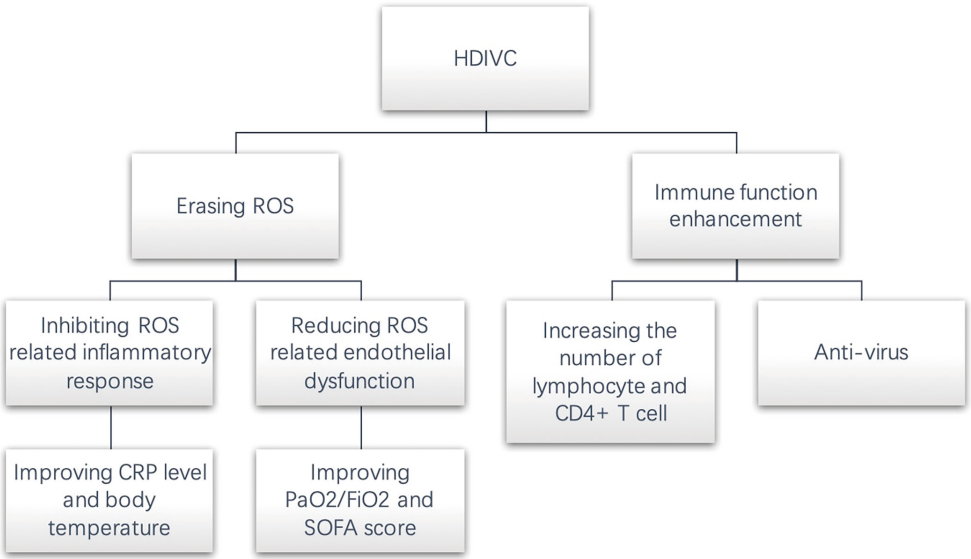

Figure S3 Flowchart of HDIVC protocol. The application of HDIVC protocol varied on the disease severity which are classified into mild, moderate, severe and critical referred to the guideline of National Health and Family Planning Commission of the People's Republic of China (the seventh edition). The mild type does not need HDIVC treatment. The HDIVC for moderate, severe and critical type are mainly divided into two parts, one is the routine usage of HDIVC from admission and last for 7 days, the other is the rescue therapy after aggravation occurs, which means disease severity transfer to the worse level within 24 to 48 hours. This protocol was promoted based on the previous clinical practice and research of Shanghai COVID-19 pneumonia expert group. HDIVC, high dose intravenous vitamin C; COVID-19, coronavirus disease 2019.

http://dx.doi.org/10.21037/apm-20-1387