Treatment of COVID-19 Patients with Prolonged Post-Symptomatic Viral Shedding with Leflunomide -- a Single-Center, Randomized, Controlled Clinical Trial

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summary

Leflunomide has inhibitory efficacy on SARS-CoV-2 replication in vitro. However, our data show that it did not shorten the number of virus clearance days or shorten the duration of stay in the hospital for COVID-19 patients with prolonged post-symptomatic shedding.

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Abstract

Objective: To evaluate the efficacy and safety of leflunomide, an approved dihydroorotate dehydrogenase inhibitor, to treat COVID-19 patients with prolonged post-symptomatic viral shedding.

Methods: We conducted a prospective, randomized, controlled, open-label trial involving hospitalized adult COVID-19 patients with prolonged PCR positivity. Patients were randomly assigned to receive either leflunomide (50 mg, q12h, three consecutive times, orally; then 20 mg, once daily for 8 days), in addition to nebulized interferon alpha 2a (IFN α-2a, 3 million IU each time, twice daily for 10 days), or nebulized IFN α-2a alone for 10 days. The primary end point was the duration of viral shedding.

Results: A total of 50 COVID-19 patients with prolonged PCR positivity were randomized into 2 groups; 26 were assigned to the leflunomide group, and 24 were assigned to the interferon alone group. Treatment with leflunomide was not associated with a difference from the interferon alone group in the duration of viral shedding (hazard ratio for negative RT-PCR, 0.70; 95% confidence interval, 0.391-1.256; *P*=0.186). In addition, the patients given leflunomide did not have a substantially shorter length of hospital stay than patients treated with interferon alone, with median (IQRs) durations of 29.0 (19.3-47.3) days and 33.0 (29.3-42.8) days, respectively, *P*=0.170. Two leflunomide recipients were unable to complete the full 10-day course of administration due to adverse events.

Conclusions: In COVID-19 patients with prolonged PCR positivity, no benefit in terms of the duration of viral shedding was observed with the combined treatment of leflunomide and IFN α-2a beyond IFN α-2a alone.

Key words: COVID-19; SARS-CoV-2; leflunomide; interferon alpha 2a; viral shedding

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INTRODUCTION

Although clinical trials of compassionate or off-label uses of several drugs have been conducted, there is no specific and effective medication to treat patients with COVID-19 [1,2,3]. Partial clinical trial results of lopinavir-ritonavir, remdesivir, chloroquine and hydroxychloroquine have already been performed in different countries, but have shown only moderate and controversial effects [2, 4]. Therefore, it is still necessary to seek safe and solid strategies to treat COVID-19 when facing the increasing number of patients worldwide [5].

The pandemic of COVID-19 has been under control in Wuhan, China since March, 2020, but some patients remained viral RNA-positive after their symptoms had resolved and their abnormal CT imaging had improved significantly [6, 7, 8]. Long-term COVID-19 positive patients cause many problems [9], for example, they have to stay in the hospital for a long time and require more medical resources. In addition, they often had psychological disorders. Moreover, no specific therapeutic agents have been recommended for COVID-19 patients with prolonged post-symptomatic shedding [10], which has become a great concern [11].

Acute RNA virus replication, including SARS-CoV-2, largely depends on intracellular pyrimidine resources, and antagonists of dihydroorotate dehydrogenase (DHODH), a rate-limiting enzyme in the fourth step of the *de novo* pyrimidine biosynthesis pathway, can efficiently prohibit viral genome replication in infected cells [12]. Leflunomide, an approved DHODH inhibitor, has been widely used to treat patients with autoimmune diseases [13], but whether leflunomide can be used to treat COVID-19 patients is unknown. As COVID-19 patients also suffer from excessive inflammations similar to autoimmune patients [14], leflunomide may benefit COVID-19 patients through its antiviral and antiinflammation effects. A small-scale study of leflunomide treatment for confirmed patients with COVID-19 was conducted by our team, in which, leflunomide resulted in beneficial virologic clearance and length of hospital stay [15]. Based on that background, we conducted a prospective randomized, controlled, open-label trial, to evaluate the efficacy and safety of oral leflunomide to treat hospitalized COVID-19 patients with prolonged post-symptomatic viral shedding.

METHODS

Patients

From March 10, 2020 to April 12, 2020, a total of 50 consecutive patients with confirmed COVID-19 with prolonged viral shedding were enrolled as study candidates. All patients were referred from other COVID-19 designated wards or makeshift (Fangcang) hospitals to the East Campus, Renmin Hospital of Wuhan University.

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The inclusion criteria were as follows: (1) aged 18 -70 years with a diagnosis of COVID-19 conforming to *the Chinese Guidelines* [16]; (2) hospitalized for prolonged post-symptomatic viral shedding; (3) able to orally take medication; (4) non-pregnant women; (5) effective contraception for 7 days after taking the last medication. Candidates were excluded based on the following: (1) presence of any condition that would not allow the protocol to be followed, including known allergy to leflunomide, use of medications that are contraindicated with leflunomide and that could not be replaced or stopped during the trial period; (2) pregnant or breast-feeding; (3) known other serious comorbidities, such as liver, cardiovascular, cerebrovascular diseases, severe renal insufficiency or advanced cancer; (5) had received interferon before enrollment; (6) unwilling to participate in the study.

Ethics approval and consent to participate

This clinical trial received approval from the Ethics Committee of the Renmin Hospital of Wuhan University (No.WDRY2020-K063) and written informed consent was obtained from each participant. The study was registered at the Chinese Clinical Trial Registry (ChiCTR 2000030058).

Trial design and study protocol

Patients were assessed for eligibility on the basis of the inclusion and exclusion criteria (Figure 1). At the first interview, each candidate completed a comprehensive questionnaire including demographics, comorbidities, initial-episode syndromes and disease severity at the first admission, length of virus shedding from onset to enrollment, duration of post-symptomatic viral shedding, antiviral medication before enrollment,etc.However,the original protocol had been amended,which was for a multicenter, randomized, double-blind, controlled clinical trial. Due to few new COVID-19 patients in Wuhan, China since early March 2020, only convalescing patients with prolonged post-symptomatic viral shedding rather than those in the acute stage were enrolled in single center,with a small sample size.

Fifty eligible patients were randomly assigned to a combination treatment group that received leflunomide (50 mg, q12h, three consecutive times, orally; then 20 mg, once a day for 8 days; a total course of 10 days) plus nebulized IFN α-2a (3 million IU each time, adding 2 ml of sterilized water, atomization inhalation twice daily for 10 days), or to a control group that received nebulized IFN α-2a alone for 10 days. Leflunomide tablets (10 mg per tablet) were produced by Long March-Xinkai Pharmaceutical Co., Ltd, Suzhou, China. Recombinant human IFN α-2a solution (3 million IU/ml) for nebulization was produced by 3SBIO Inc., Shenyang, China.

This was an open-label, prospective randomized, controlled trial, which was conducted at East Campus, Renmin Hospital of Wuhan University. The enrollment was initiated on March 10, 2020 and ended on April 12, 2020. The last patient studied was discharged on April 26, 2020 and was followed-up until May 25, 2020.

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Criteria for prolonged post-symptomatic viral shedding

Since there is no standard definition, we adopted the following definition of COVID-19 patients with prolonged post-symptomatic viral shedding, which refers to laboratory confirmed patients with COVID-19 who continued to have nasopharyngeal RT-PCR positivity at least two weeks after symptom resolution and after their abnormal CT imaging improved significantly.

Measurement of virus shedding by RT-PCR

After enrollment, serial nasopharyngeal swab specimens were obtained at the baseline (before leflunomide or IFN α-2a was administered) and once every two days until nucleic acid tests were negative twice consecutively with an interval of $\geq$24 hours. RT-PCR for SARS-CoV-2 was performed using a commercial kit (GeneoDx Biotech Co., Ltd, Shanghai, China).

Clinical and laboratory monitoring

Clinical symptoms of patients were assessed once daily by trained nurses using diary cards, analysis of peripheral blood cells, biochemical indicators and chest imaging studies performed at the baseline, on day 3, one day after treatment and or one day before discharge for patients meeting discharge criteria within ten days of enrollment. Data were recorded on paper case record forms, then were entered into an electronic database and validated by the clinical trial staff.

Discharge criteria and follow-up after discharge

Discharge criteria were as follows [16]: having a normal temperature for >3 days, significant improvements of respiratory symptoms and CT imaging, nucleic acid tests negative twice consecutively with an interval of $\geq$24 hours. After discharge, the patients were isolated at a designated place for 14 days as recommended [16], which was arranged by community committees where the patients resided. They were followed-up by primary health-care facilities and were re-tested for viral nucleic acid on days 7 and 14. After that, they stayed in their homes for a second isolation period of 14 days, and were then retested for viral nucleic acid by the end of this quarantine period. We collected each patient's medical information during the isolation, which was shared with permission. In our study, enrolled patients with five consecutively negative nucleic acid tests were considered as having "true negative" results (two times during hospitalization, two times during the first isolation, and one time at the end of the second quarantine). If any patient at any time-point had a positive test for SARS-CoV-2, they were sent to a designated site for isolation and medical observation.

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Outcome measures

The primary end point was the duration of viral shedding, which was defined as the time from randomization to the first negative nucleic acid test of five consecutive RT-PCR results. Other clinical outcomes included clinical status, i.e. progressive rate to severe illness, syndromes, peripheral blood cells and biochemical parameters, C-reactive protein and inflammatory cytokines, length of hospital stay, etc. Safety outcomes included adverse events that occurred during treatment, serious adverse events, and premature discontinuation of treatment.

Statistical analysis

Continuous variables are presented as medians (IQR). The normality of the distribution of variables was performed using the Kolmogorov-Smirnov test and statistical comparisons using a t-test. Categorical variables are expressed as absolute numbers or percentages and are compared by the χ^2 test, Fisher's exact test or one-way ANOVA. The time to negative RT-PCR test was developed using the Kaplan-Meier method and was compared with a log-rank test. A P<0.05 was considered statistically significant. All statistical analyses were performed using SAS 9.4 software (SAS Institute Inc, Cary, NC).

RESULTS

The characteristics of the patients in this study are summarized in Tables 1 and 2. Of the 50 patients who underwent randomization and treatment assignment, 26 were assigned to the combination treatment group that orally received leflunomide plus nebulized IFN α-2a, and 24 were assigned to the control group that received nebulized IFN α-2a alone. In the combination treatment group, 24 patients (92.3%) received all treatments as assigned, but two patients did not complete the 10 day treatment regimen, one due to serious diarrhea 2 days after taking the drug, and the other due to impaired liver function. There were no significant differences in age [56.0 (43.0-67.3) vs 55.5 (47.8-66.5), P=0.836] or gender [13:11 vs 9:15 (M:F), P=0.274] between the combination treatment group and the control group. In addition, no significant differences were found between the two groups in terms of most first symptoms, disease severity at first admission, comorbidities, the use of anti-viral drugs, duration of post-symptomatic viral shedding or length from disease onset to enrollment [44.5 (30.0-47.8) vs. 44.0 (36.3-52.0) days, P=0.536]. Regarding the first symptoms, more cough and expectoration was found in the combination treatment group. However, the differences of initial symptoms did not influence the lengths of virus shedding between the two groups (Tables 1 and 3).

At enrollment, none of the patients in either group had a fever or respiratory symptoms, and none had obvious infiltrative lesions on pulmonary imaging. The median interval time between the

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disappearance of symptoms and randomization was 26.0 days (IQR, 15.0 to 33.0 days) in the combination treatment group and 24.0 days (IQR, 17.0 to 32.0 days) in the control group (Table 1). There were no important between-group differences in baseline laboratory test results at enrollment, except for the level of creatine kinase in the control group, the level of tumor necrosis factor in the combination group was slightly higher, although both were within the normal range (Table 2).

Twenty-four of the 26 patients in the combination treatment group and all 24 patients in the control group completed this study and were discharged. No deaths or severe illness occurred and the illness severity was not worse in either group. In terms of the duration of viral shedding after treatment, patients assigned to the combination treatment group had a time to negative RT-PCR results that was not different from patients assigned to the control group (Figure 2), the median time was 8.0 (6.0-15.5) days and 11.5 (6.3-16.5) days, respectively, P=0.488. In addition, patients in the combination treatment group had a similar duration from randomization to hospital discharge as the control group (median, 29.0 days vs. 33.0 days; P=0.170).

Laboratory examinations were conducted before and after treatment for all patients (Table 2). Of the post-treatment test results, there were no differences between the two groups except that the lymphocyte count in the control group was slightly higher than in the combination treatment group [1.7 (1.1-2.3) $\times 10^9$/L vs.1.4 (1.0-1.7) $\times 10^9$/L, P=0.045].

In terms of comparison between pre- and post- treatment, the control group had a mild decrease after treatment in their levels of creatine kinase, urea, creatinine, LDH, fibrinogen, prothrombin time and APTT. In addition, the titration of IgM for SARS-Cov-2 was also lower [(10.9 (4.5-44.6) vs. 4.4 (2.8-54.2), AU/mL, P= 0.017)]. However, compared with pre-treatment, there was not a significant decrease in the levels of C-reactive protein or LDH in the combination treatment group after treatment. Further, the combination treatment group did not have significantly reduced levels of inflammatory cytokines, i.e. IL-2, IL-4, IL-6, IL-10, TNF or interferon-γ. The titration of IgM for SARS-Cov-2 was also not changed much, although the creatine kinase level and the prothrombin time decreased.

For safety, a total of 10 patients in the combination treatment group and 4 in the control group reported adverse events (Table 3) but that was not significantly different between the two groups (41.7% vs. 16.7%, P=0.057). There was one serious gastrointestinal adverse event that caused the discontinuation of treatment in the combination treatment group but none occurred in the control group, which was judged by the investigators to be related to the trial medication. For laboratory results, the absolute number of increased liver enzymes in the combination treatment group was higher than in the control group but was not statistically different (Table 2), although one patient in the combination treatment group discontinued treatment on day 3 due to liver enzymes (ALT 109 U/L, AST 51 U/L). Other obvious laboratory abnormalities were not observed.

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DISCUSSION

This randomized trial found that leflunomide treatment added to nebulized IFN α-2a was not associated with improvement of viral negative conversion in COVID-19 patients with prolonged post-symptomatic shedding, and the between-group difference in the median time to negative virus nucleic acid (median,8.0 days vs.11.5 days) was not significant.

Persistent viral shedding is a serious problem [17]. Cao and colleagues reported that SARS-CoV-2 RNA was detected in 40.7% of their patients on day 28 after a 14-day treatment regimen with lopinavir-ritonavir [18]. Another report showed that the median duration of viral shedding was 20 days in patients with COVID-19 and could be as long as 37 days [19]. An analysis of the transmission of COVID-19 revealed that 86% of subjects in China in January-February 2020 potentially contracted the virus from patients with no or minimal symptoms [20]. The prolonged existence of virus presents difficulties in attempts to control the community spread of SARS-CoV-2.

Partial in vitro studies or clinical trials have suggested the potential therapeutic activity of several compounds against coronaviruses [21], however, there are no specific antiviral pharmaceutical treatments available for patients with COVID-19 [22]. The results of those studies did not show clinical improvement or the clinical trial results were controversial, including lopinavir-ritonavir [18], remdesivir [23], favipiravir [24] and chloroquine or hydroxychloroquine [25].

We evaluated the efficacy and safety of leflunomide on SARS-CoV-2 infection in this study and compared it with the roles of interferon treatment alone. Interferon is recommended to be used for patients with COVID-19 by the Chinese guidelines [16], for it has broad-spectrum antiviral activity [26], has been widely used for the treatment of virus infections [26, 27, 28], and is also effective for treating patients with COVID-19 [29, 30]. Leflunomide is capable of inhibiting viral RNA genome replication and rescues mice from advanced influenza infections [12]. Leflunomide directly targets DHODH, the host's *de-novo* pyrimidine synthesis enzyme, to cut off intercellular pyrimidine resources required as the starting step of building the viral RNA genome [12]. Like chloroquine and hydroxychloroquine, leflunomide has a dual mechanism of antiviral and immunoregulation and has been approved to treat arthritis for many years [31, 32]. Leflunomide has a clear-cut drug target of DHODH and has few off-target effects [33], whereas chloroquine and hydroxychloroquine are multi-targeted and have more severe adverse effects [34]. Therefore, DHODH inhibitors may be attractive drugs for treating acute and severe virus infection diseases [35]. In a preliminary trial, we found that leflunomide resulted in beneficial virologic clearance and length of hospital stay for patients with COVID-19 [15].

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In the present study, the baseline characteristics of the patients at enrollment were generally balanced across the two groups that did not differ with regard to duration, severity of illness and majority baseline laboratory results. However, differences in the negative conversion of virus nucleic acid between the combination treatment group and the control group were not observed. As compared to treatment with nebulized IFN α-2a only, the combination of oral leflunomide and nebulized IFN α-2a did not significantly shorten the duration of viral shedding time or the duration from randomization to hospital discharge. The results indicate that leflunomide did not accelerate virus clearance in COVID-19 patients with prolonged positive nucleic acid testing. Remarkably, the combination therapy did not significantly reduce levels of C-reactive protein, LDH or inflammatory cytokines. The titration of IgM for SARS-Cov-2 was not changed much, although the level of creatine kinase and the prothrombin time decreased after treatment. In contrast, nebulized treatment with IFN α-2a only decreased the levels of creatine kinase, LDH, fibrinogen, prothrombin time, APTT and the titration of IgM for SARS-Cov-2.

For safety, two leflunomide recipients discontinued treatment due to gastrointestinal adverse events or abnormal liver function, however, there was no statistical difference in the total number of adverse events between the two groups. The side-effect profile observed in the current trial arouses concern about the use of higher or more prolonged leflunomide dose regimens in efforts to improve outcomes.

Our trial has several limitations. Particularly, the limited sample size, as well as the unblinded nature of the trial and the lack of a placebo were the main shortcomings. Due to the few new COVID-19 patients in Wuhan, China since early March 2020, convalescing patients rather than those in the acute stage were enrolled, which may cause a bias in evaluating the potential effectiveness of leflunomide on COVID-19 infections. The inhibition of DHODH may mainly hinder the activated, fast proliferating/replication of immune cells/viruses that require the *de novo* synthesis of pyrimidine bases, whereas resting cells are less affected since pyrimidine bases can be recruited by the salvage pathway [36]. However, the question of whether earlier leflunomide treatment of patients with acute COVID-19 could have clinical benefit is an important one that requires further studies. Because common antirheumatic drugs have not shown consistent antiviral effects as expected [37, 38], it is considered prudent to continue any ongoing immunosuppressive therapy [39].

Conclusions

Our data show that leflunomide did not shorten the number of virus clearance days or shorten the duration of stay in the hospital for COVID-19 patients with prolonged post-symptomatic shedding. With a larger sample, double-blinded and controlled design, future trials may help to clarify the antiviral efficacy and drug safety of leflunomide.

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Contributors

M.M.W., Y.Z., W.H.H., D.Z., Y.T.Z., T.W., Z.S.Z., X.C.L. and S.L.Z. collected the epidemiological and clinical data. M.M.W., Y.Z. and W.H.H. were responsible for enrollment and clinical monitoring. Z.L.L., L.L. and Z.H.W were responsible for the distribution and storage of medicines. W.H.H. and D.Z. were responsible for statistical data. M.M.W., Y.Z and K.H. drafted the manuscript. K.H. was responsible for funding, study conception and design, revising and submitting the final manuscript.

Acknowledgments

We respectfully thank all patients enrolled in this study. This work was supported by the National Key Research and Development Plan for the Emergency Management of Novel Coronavirus Pneumonia,China (No.2020YFC0845100) and the Wuhan Municipal Key Technology Project on Novel Coronavirus Pneumonia, China (No.2020020101010005).

Role of the Funder/Sponsor

The funding agencies had no role in the study design and clinical medications; collection, analysis, and interpretation of the data; preparation, written, review, or approval of the manuscript.

Conflict of Interest Disclosures

Zhao Y and Hu WH contributed equally with Wang MM. The authors have no competing interest to declare for this study.

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Table 1 Demographics, first-episode syndromes and comorbidities in patients with confirmed COVID-19 at the first admission [Median (IQR) or n]

Lianhua Qingwen capsule is a kind of Chinese traditional medicine and is recommended for patients with COVID-19 [16].

Combination group: leflunomide plus IFN α-2a; Control group: IFN α-2a alone.

Table 2 Laboratory results of patients with COVID-19 at enrollment and after treatment [Median (IQR) or n]

Combination group: leflunomide plus IFN α-2a; Control group: IFN α-2a alone.

WBC = White blood cell count; N = Neutrophil count; L = Lymphocyte count; CRP = C-reactive protein; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; LDH = Lactate dehydrogenase; Ultra-TnI = Ultratroponin I; APTT = activated partial thromboplastin time; IL = Interleukin; TNF = tumor necrosis factor.

a: Comparison of baseline data between the two groups.

b: Data comparison between after treatment the two groups.

Table 3 Outcomes and adverse events of patients with COVID-19 after enrollment [Median (IQR) or n]

Combination group: leflunomide plus IFN α-2a; Control group: IFN α-2a alone.

Figure 1. Study flow chart.

Figure 2. Kaplan–Meier curve showing time to negative test for patients who were treated with leflunomide and patients who were treated with interferon-α only.

Compared with interferon α-2a nebulized therapy, oral leflunomide did not significantly shorten the time of virus shedding in patients with SARS-Cov-2 infection (hazard ratio for negative RT-PCR,0.70; 95% confidence interval [CI], 0.391-1.256; P=0.186).

Combination group: leflunomide plus IFN α-2a; Control group: IFN α-2a alone.

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Table 1 Demographics, first-episode syndromes and comorbidities in patients with confirmed COVID-19 at the first admission [Median (IQR) or n]

Parameters	Combination group (N=24)	Control group (N=24)	*P* value
Demographics			
Age	56.0 (43.0-67.3)	55.5 (47.8-66.5)	0.836
Sex (M:F)	13:11	9:15	0.274
First symptoms			
Fever (T ≥37.0℃), n (%)	18 (75.0)	17 (70.8)	0.745
Tmax,℃ (IQR)	38.0 (37.0-38.9)	38.3 (37.0-38.6)	0.741
Cough, n (%)	17 (70.8)	8 (33.3)	0.009
Expectoration, n (%)	8 (33.3)	2 (8.3)	0.033
Chest tightness, n (%)	7 (29.2)	7 (29.2)	1.000
Fatigue, n (%)	8 (33.3)	10 (41.7)	0.551
Myalgia, n (%)	6 (25.0)	2 (8.3)	0.245
Nausea, n (%)	0 (0)	1 (4.2)	1.000
Vomiting, n (%)	0 (0)	2 (8.3)	0.470
Diarrhea, n (%)	1 (4.2)	2 (8.3)	1.000
Poor appetite, n (%)	4 (16.7)	1 (4.2)	0.345
Severity on first admission			
Mild, n (%)	11 (45.8)	10 (41.7)	0.979
Moderate, n (%)	9 (37.5)	9 (37.5)	
Severe, n (%)	3 (12.5)	4 (16.7)	
Critical, n (%)	1 (4.2)	1 (4.2)	
Comorbidity			
Hypertension, n (%)	7 (29.2)	5 (20.8)	0.505
Diabetes, n (%)	0 (0)	2 (8.4)	0.470
Coronary artery disease, n (%)	1 (4.2)	0 (0)	1.000
Chronic obstructive pulmonary disease, n (%)	1 (4.2)	1 (4.2)	1.000
Malignancy, n (%)	1 (4.2)	0 (0)	1.000
Chronic liver disease, n (%)	1 (4.2)	0 (0)	1.000
Length of virus shedding from onset to enrollment, day	44.5 (30.0-47.8)	44.0 (36.3-52.0)	0.536
Patients with initial cough and expectoration, day*	54 (50.0-61.0)	42.0 (41.0-53.8)	0.096

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Duration of post-symptomatic virus shedding, day	26.0 (15.0-33.0)	24.0 (17.0-32.0)	0.549
Patients with initial cough and expectoration, day※	18.5 (11.0-23.25)	30.0 (9.5-37.5)	0.116
Anti-viral drugs before enrollment			
Lopinavir - Ritonavir, n (%)	1(4.2)	4 (16.7)	0.345
Arbidol Hydrochloride, n (%)	17 (70.8)	16 (66.7)	0.755
Oseltamivir, n (%)	4 (16.7)	3 (12.5)	1.000
Ribavirin, n (%)	7 (29.2)	9 (37.5)	0.540
Lianhua Qingwen capsule, n (%) #	14 (58.3)	14 (58.3)	1.000
Hydroxychloroquine, n (%)	12 (50.0)	13 (54.2)	0.773
Thymosin Alph-1enteric coated tablet, n (%)	14 (58.3)	14 (58.3)	1.000
Immunoglobulin, n (%)	3 (12.5)	6 (25.0)	0.460
Glucocorticoid, n (%)	8 (33.3)	7 (29.2)	0.755
Antibiotics, n (%)	16 (66.7)	12 (50.0)	0.242

Combination group: leflunomide plus IFN α-2a. Control group: IFN α-2a alone.

✻ Length of virus shedding from onset to enrollment in patients with initial cough and expectoration.

※ Patients with initial cough and expectoration in patients with initial cough and expectoration.

Lianhua Qingwen capsule is a kind of Chinese traditional medicine and is recommended for patients with COVID-19 [16].

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Table 2 Laboratory results of patients with COVID-19 at enrollment and after treatment [Median (IQR) or n]

Parameters (normal range)	**Combination group** [n=24, Median (IQR) or n]			**Control group** [n=24, Median (IQR) or n]			*P*-value [a]	*P*-value [b]
	Baseline	After treatment	*P*-Value	Baseline	After treatment	*P*-Value		
WBC (3.5-9.5×10^9/L)	5.0 (4.3-6.6)	5.9 (4.2-7.8)	0.122	5.6 (4.7-7.3)	5.6 (5.0-7.6)	0.372	0.257	0.822
WBC<4.0	4 (16.7)	5 (20.8)	1.000	4 (16.7)	3 (12.5)	1.000	1.000	0.699
N (1.8-6.3,×10^9 /L)	2.8 (2.3-3.9)	3.9 (2.0-5.5)	0.131	3.3 (2.5-4.4)	3.6 (2.4-4.4)	0.948	0.386	0.693
L (1.1-3.2×10^9 /L)	1.5 (0.9-1.7)	1.4 (1.0-1.7)	0.889	1.5 (1.0-2.0)	1.7 (1.1-2.3)	0.093	0.464	0.045
L<1.0	7 (29.2)	6 (25.0)	1.000	6 (25.0)	1 (4.2)	0.102	1.000	0.102
Haemoglobin (130 - 175 g/L)	123.5 (114.8-144.0)	127.0 (118-137.0)	0.976	123.0 (109.5-140.8)	122 (110-134.5)	0.574	0.592	0.217
Platelet count (125-350×10^9/L)	201.5 (172.0-234.0)	201.0 (168.0-252)	0.808	206.5 (161.8-254.3)	200.0 (174.0-249.3)	0.663	0.650	0.888
CRP (0-10 mg/L)	5.0 (5.0-19.1)	5.0 (5.0-12.0)	0.463	8.4 (5.0-34.4)	5.0 (5.0-18.9)	0.064	0.592	0.777
Albumin (40 - 55 g/L)	41.9 (38.5-42.8)	41.1 (38.2-41.1)	0.862	42.4 (35.9-44.6)	41.8 (40.3-44.2)	0.061	0.657	0.297
ALT (9- 50 U/L)	23.5 (15.5-35.5)	27.0 (16.0-86.5)	0.122	28.5 (18.3-53.0)	29.0 (13.5-53.5)	0.679	0.477	0.659
AST (15 - 40 U/L)	22.5 (18.0-32.3)	22.0 (18.0-31.5)	0.663	26.0 (17.3-38.0)	22.0 (18.5-35.5)	0.289	0.496	0.860
Alkaline phosphatase (45-125U/L)	65.5 (56.3-73.0)	66.0 (49.5-85.5)	0.667	65.5 (45.0-77.5)	56.0 (51.0-78.0)	0.554	0.918	0.394
Bilirubin (0-23 mmol/L)	12.6 (9.3-15.8)	12.5 (10.2-17.0)	0.322	10.1 (7.0-14.5)	10.2 (7.4-14.1)	0.136	0.081	0.097
Potassium (3.5 - 5.3 mmol/L)	4.0 (3.6-4.2)	4.0 (3.8-4.2)	0.189	3.8 (3.4-4.2)	4.1 (3.9-4.3)	0.139	0.477	0.593
Sodium (137 - 147, mmol/L	140.0 (136.3-143.0)	141.5 (138.3-146.0)	0.185	140.0 (137.0-144.8)	142.0 (139.0-145.3)	0.081	0.489	0.565
Urea (3.6 - 9.5 mmol/L)	4.3 (3.2-5.8)	4.8 (4.2-5.7)	0.639	5.5 (4.5-6.1)	4.6 (4.0-5.2)	0.002	0.085	0.378
Creatinine (57-111 µmol/L)	59.0 (49.3-75.5)	54.0 (47.0-73.0)	0.088	52.0 (45.0-66.3)	52.0 (46.5-58.0)	0.003	0.054	0.518
Creatine kinase (18-198 U/L)	57.5 (33.8-75.0)	39.0 (27.3-58.3)	0.019	86.5 (49.3-119.5)	44.0 (27.5-80.5)	0.001	0.037	0.573
LDH (120 - 250 U/L)	195.0 (167.0-233.8)	195.5 (172.8-132.8)	0.156	210.0 (171.0-285.5)	185.0 (170.0-205.0)	0.009	0.483	0.272
Ultra-TnI (0-0.04 ng/ml)	0.006 (0.006-0.006)	0.006 (0.006-0.006)	1.000	0.006 (0.006-0.007)	0.006 (0.006-0.007)	0.285	0.833	0.874
D-dimer (0 - 0.55 mg/L)	0.4 (0.2-0.7)	0.3 (0.2-0.7)	0.206	0.4 (0.2-1.4)	0.4 (0.2-1.3)	0.865	0.602	0.274
Fibrinogen (2 - 4 g/ dL)	2.8 (2.3-3.2)	2.9 (2.3-3.2)	0.073	3.1 (2.7-4.4)	3.1 (2.7-3.3)	0.048	0.663	0.127
Prothrombin time (9 - 13 s)	11.2 (10.9-12.0)	11.2 (10.8-11.8)	0.042	11.1 (10.8-12.3)	11.4 (10.4-11.7)	0.012	0.550	0.140
APTT (25 - 31.3 s)	27.0 (25.1-29.0)	26.7 (24.9-28.8)	0.913	27.9 (25.4-30.3)	26.8 (24.2-27.6)	0.003	0.233	0.498
IL-2 (≤11.4 pg/mL)	3.6 (3.2-3.8)	3.8 (3.5-3.8)	0.799	3.5 (3.2-3.7)	3.7 (3.5-3.8)	0.248	0.133	0.876
IL-4 (≤12.9 pg/mL)	3.1 (2.9-3.4)	3.0 (2.8-3.4)	0.533	3.2 (2.7-3.6)	3.0 (2.9-3.6)	0.433	0.838	0.758
IL-6 (≤20.0 pg/mL)	6.0 (4.8-17.2)	6.4 (5.2-8.8)	0.861	6.0 (4.3-13.6)	6.7 (4.5-9.6)	0.075	0.510	0.878
IL-10 (≤5.9 pg/mL)	5.9 (5.0-7.3)	6.1 (5.4-8.40	0.721	5.7 (4.9-6.3)	6.3 (5.4-7.9)	0.477	0.625	0.902

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TNF (≤5.5pg/mL)	3.6 (3.1-4.4)	3.3 (3.2-3.5)	0.959	3.2 (2.8-3.6)	3.2 (2.7-4.0)	0.456	0.038	0.644
Interferon-γ (≤18 pg/mL)	3.3 (2.9-4.3)	3.3 (3.1-3.6)	0.756	3.2 (3.0-3.4)	3.4 (3.0-3.6)	0.937	0.460	0.829
IgG for SARS-Cov-2 (＜10 AU/mL)	146.3 (74.0-359.8)	134.4 (72.0-187.0)	0.674	186.6 (132.6-247.5)	187.3 (115.5-445.4)	0.093	0.245	0.208
IgM for SARS-Cov-2 (＜10 AU/mL)	20.0 (6.8-76.6)	27.6 (2.3-107.0)	0.141	10.9 (4.5-44.6)	4.4 (2.8-54.2)	0.017	0.391	0.753

Combination group: leflunomide plus IFN α-2a; Control group: IFN α-2a alone.

WBC = White blood cell count; N = Neutrophil count; L = Lymphocyte count; CRP = C-reactive protein; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; LDH = Lactate dehydrogenase; Ultra-TnI = Ultratroponin I; APTT = activated partial thromboplastin time; IL = Interleukin; TNF = tumor necrosis factor.

a: Comparison of baseline data between the two groups.

b: Data comparison between after treatment the two groups.

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Table 3 Outcomes and adverse events in patients with COVID-19 after enrollment [Median (IQR) or n]

Parameters	**Combination group** (N=24)	**Control group** (N=24)	*P* value
Duration of treatment, day	10.0 (7.0-12.0)	10 (7.0-12.0)	1.000
Conversion to severe case after enrollment, n (%)	0 (0)	0 (0)	-
Death after enrollment, n (%)	0 (0)	0 (0)	-
Duration of viral shedding after enrollment, day	8.0 (6-15.5)	11.5 (6.3-16.5)	0.488
Patients with initial cough and expectoration, day※	11.0 (7.0-16.0)	16.0 (7.5-38.8)	0.559
Length of hospital stay, day	29.0 (19.3-47.3)	33.0 (29.3-42.8)	0.170
Side effects after enrollment, n (%)	10 (41.7)	4 (16.7)	0.057
Symptoms			
Nausea, n (%)	2 (8.3)	1 (4.2)	1.000
Vomiting, n (%)	1 (4.2)	1 (4.2)	1.000
Diarrhea, n (%)	0 (0)	1 (4.2)	1.000
Stomach ache, n (%)	1 (4.2)	0 (0)	1.000
Dry mouth, n (%)	1 (4.2)	0 (0)	1.000
Chest tightness, n (%)	2 (8.3)	0 (0)	0.470
Palpitations, n (%)	1 (4.2)	1 (4.2)	1.000
Insomnia or sleep disturbances, n (%)	2 (8.3)	1 (4.2)	1.000
Abnormal laboratory results			
Leukopenia, n (%)	1 (4.2)	1 (4.2)	1.000
Lymphopenia, n (%)	2 (8.3)	0 (0)	0.470
Thrombocytopenia, n (%)	1 (4.2)	1 (4.2)	1.000
Anemia, n (%)	3 (12.5)	1 (4.2)	0.602
Increased AST, n (%)	3 (12.5)	0 (0)	0.233
Increased ALT, n (%)	7 (29.2)	1 (4.2)	0.053
Hypoalbuminemia, n (%)	1 (4.2)	0 (0)	1.000

Combination group: leflunomide plus IFN α-2a; Control group: IFN α-2a alone.

※ Duration of viral shedding after enrollment in patients with initial cough and expectoration.

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Figure 1

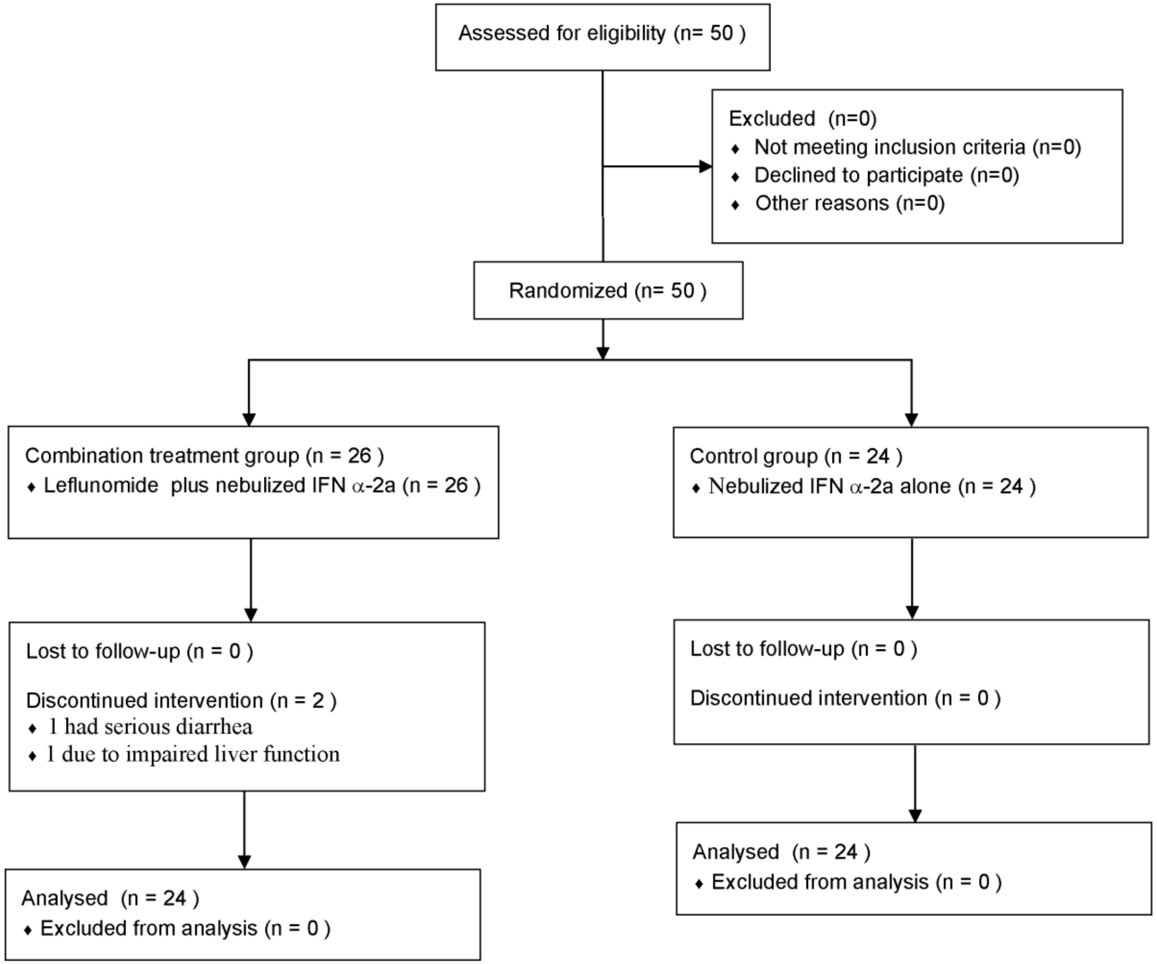

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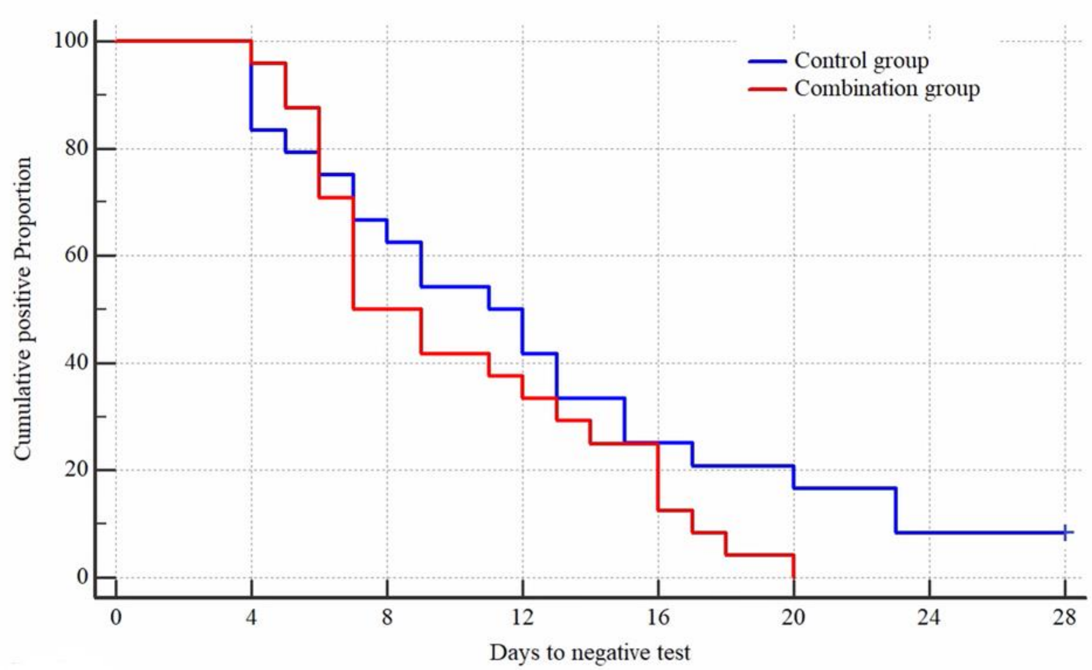
Figure 2



No. at risk								
Combination group	24	23	12	8	3	0		
Control group	24	20	15	10	6	4	2	2