Recombinant interleukin-2 stimulates lymphocyte recovery in severe patients with COVID-19

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Research Article

Keywords: COVID-19, recombinant human interleukin-2, lymphopenia, cytokine, C-reactive protein

DOI: https://doi.org/10.21203/rs.3.rs-40006/v1

Abstract

Object: A recently developing pneumonia called COVID-19 which caused by SARS-CoV-2 has quickly spread across the world. Lymphopenia and a proinflammatory cytokine storm frequently happened in severe COVID-19 patients. But no specific immunomodulate therapy on COVID-19 had been reported. In this retrospect case control study, we observed the potential therapeutic effect of recombinant human interleukin-2 (rIL-2) on severe COVID-19 patients in a hospital in Wuhan, China.

Methods: Fifty nine severe cases with COVID-19 admitted in hospital from January 29, 2020 to February 29, 2020 were included in this study. Twenty patients received a one-week to 10 days subcutaneous injection of the recombinant human interleulin-2 1 million IU per day other than regular treatment were classified as rIL-2 group. Twenty from thirty nine patients with regular treatment without intervention of rIL-2 were matched as the control group. Clinical characteristic such as age, gender, symptoms, signs, laboratory data and comorbidities were paired in these two groups. Changes of lymphocytes counts, IL-6 and C- reactive protein (CRP) before and after rIL-2 treatment and differences between rIL-2 group and non-rIL-2 group were analyzed.

Results: There were a clearly visible increasing in lymphocyte counts and a decreasing in CRP level in non rIL-2 group and rIL-2 group. The difference of the change of lymphocyte counts were significant in rIL-2 group and non-rIL-2 group ($p<0.01$). Though CRP decreased more in rIL-2 group, it did not show a significant difference between the two groups ($p>0.05$).

Conclusion: RIL-2 might be a prospective adjuvant therapy for severe COVID-19 patients by increasing lymphocytes number.

Introduction

The corona virus disease 2019 (COVID−19), caused by severe acute respiratory syndrome coronavirus−2 (SARS-CoV−2) has spread out in the world, posing a critical threat to global health1. In the early stage of COVID−19, patients usually manifested a normal or lowering white blood cell count in peripheral blood, a remarkable decreasing of lymphocytes count and increasing of CRP. In the advanced stage of COVID−19, numerous inflammatory cytokines such as interleukins, tumor necrosis factor and chemokines were released and attacked the immune organs and tissues. That induced inflammatory storm and led to disease progression and multiple organ failure2,3. The degree of lymphopenia and a proinflammatory cytokine storm happened more frequently in severe COVID−19 patients than in mild cases, and are associated with the disease severity4

Recently, it was reported that CD4+ T cells decreased remarkably in patients in the early recovery stage of COVID−19. It revealed that IL−2, IL−18, IL−4 and TNFSF13 might be beneficial for the recovery of COVID−19 patients5. However, the role of these cytokines might play in the therapy of COVID−19 had not known yet.

Our study applied rIL–2, an immumodulator usually used in treatment of cancer, to the therapy of COVID–19 and investigated the changes of peripheral blood white blood cells count, lymphocytes count, levels of IL–6 and CRP. Moreover, the side effect of rIL–2 was observed simultaneously. That would make a sense of the effect and safety of rIL–2 on the treatment of COVID–19.

Methods

Study design and participants

This is a retrospective single-center case control study included 59 severe cases with COVID–19. The patients were admitted to the West campus of Union Hospital, Tongji Medical College Huazhong University of Science and Technology (Wuhan, China), from January 29, 2020 to February 29, 2020. COVID–19 was diagnosed by meeting the criteria of epidemiology and clinical syndrome, chest computerized tomography (CT) and lymphocyte manifestations and confirmed by real-time reverse transcription- polymerase chain reaction (RT-PCR) detection for routine pharyngeal swab specimens according to the New Coronavirus Pneumonia Prevention and Control Program (6th edition) published by the National Health Commission of China6. The final date of follow up was April 15, 2020. Adult severe cases were diagnosed when any of the following criteria was met: (1) Respiratory distress (≧30 breaths/ min); (2) Oxygen saturation≦93% at rest; (3) Arterial partial pressure of oxygen (PaO2)/ fraction of inspired oxygen (FiO2)≦300mmHg (lmmHg = 0.133kPa). Patients who received a one-week to 10 days subcutaneous injection of the recombinant interleulin–2 one million IU per day (rIL–2, Beijing sihuan pharmaceutical factory, production batch number 20190915) treatment during hospitalization were classified as rIL–2 group. Patients who did not receive rIL–2 during hospitalization were classified as non-rIL–2 group. Propensity score-matched cohorts were created based on variables which were expected to be potential confounders associated with exposure to rIL–2, including age, gender, fever, fatigue, cough, comorbidities (hypertension, coronary heart disease, diabetes, and cerebrovascular disease) and in-hospital medications. The rIL–2 group and non-rIL–2 group were paired at 1:1 according to the propensity scores using exact matching with a caliper size of 0.05.

We used the following inclusion and exclusion criteria to determine the study population. The inclusion criteria was aged over 18 years severe COVID–19 patients admitted in the hospital. The exclusion criteria included incomplete medical records (e.g., transfer to any other hospital), pregnancy, not survive during hospitalization, not controlled fever, severe hepatic dysfunction and severe renal dysfunction. The study protocols were approved by the ethics committee of Union Hospital, Tongji Medical College Huazhong University of Science and Technology.

Data collection

Following data were collected including patient demographic information, clinical characteristics, laboratory data, history of comorbidities and therapeutic interventions during the hospitalization. The patient demographic information (age and gender) and clinical characteristics (fever, cough and fatigue)

were collected from electronic medical system. Laboratory data (blood cell count, IL- 6, CRP, hepatic function and renal function) were collected from laboratory information system. Comorbidities (hypertension, coronary heart disease, diabetes and cerebrovascular diseases) were extracted from medical history. The in-hospital medications and interventions were collected from doctor's advice. Personal health identifying information (*e.g.,* name and ID) was anonymized and each participant was given a study ID using an electronic coding system before data extraction to preserve patient privacy. Data were carefully reviewed and confirmed by experienced physicians and were double-checked to guarantee the accuracy of the data extraction procedures.

Statistical analysis

Continuous variables were expressed as median and interquartile range (IQR), and categorical variables were expressed as number and percentage (%). Statistical differences between two groups were analyzed using the Mann-Whitney test or $\chi 2$ test. A two-side α less than 0.05 was considered statistically different. Data were analyzed in SPSS Statistics (version 23.0, IBM, Armonk, NY, USA).

Results

Participants

From January 29, 2020 to to February 29, 2020, 72 adult patients were hospitalized. After excluding five death cases, three were transferred to other hospitals, two with not controlled fever, one with severe hepatic dysfunction and two with severe renal dysfunction, a total of 59 patients were included in this study. There were 20 patients accepted rIL–2 treatment. Twenty patients were chosen and paired with the rIL–2 group from the 39 patients of non-rIL–2 group (Figure 1). The clinical data of patient demographic information and history of comorbidities were collected on admission. Symptoms, signs and laboratory data were collected before and after rIL–2 treatment in rIL–2 group and at the same time point in non-rIL–2 group. The in-hospital medications and interventions were obtained during the hospitalization. The length of hospital stay were collected at the time of patients discharged from hospital.

Baseline Characteristics of the participants and between groups

As shown in Table 1, the median age of all patients was 56 years (48, 64), ranging from 23 years to 88 years, among whom 54.24% were male. Comorbidities such as hypertension (28.81%), diabetes (15.25%), coronary heart disease (6.78%) and cerebrovascular disease (10.17%) were common. The symptoms before rIL–2 intervention of fatigue (49.15%) and cough (77.97%) were observed in most patients. There were 69.49% patients with fever on admission. After treatment no patient included in the study had fever. All patients' white blood cell counted in a median of 5.90(4.2□7.2)×109/L, neutrophil in a median of 3.98□2.8□5.1□×109/L and lymphocyte in a median of 0.980□0.8□1.4□×109/L. High serum IL–6 level with a median of 6.420 (1.5□16.2) pg/mL and high CRP with a median of 27.220 (4.7□48.2) mg/L were

demonstrated. During hospitalization, 53 (89.83%) patients accepted antiviral drugs therapy, 44 (74.58%) patients accepted antibiotics drugs therapy, 5 (10.17%) patients accepted systemic corticosteroids therapy and 56 (94.92%) patients accepted traditional Chinese medicine therapy. The above clinical characteristics in both groups before and after matching did not have significant difference.

Comparison of laboratory results in rIL–2 group patients before and after rIL–2 treatment

An obvious rising of lymphocyte count was detected after rIL–2 plus regular treatment, which was 1.935 (1.6, 3.0)×109/L compared to 1.110 (0.9, 1.4)×109/L before rIL–2 treatment in rIL–2 group (p<0.01). The CRP showed a significant decreasing after rIL–2 plus regular treatment, which lowered from 21.740 (0.5, 65.1) mg/L to 3.965 (1.5, 5.7) mg/L (p<0.05). The WBC count were 6.215 (5.4, 7.2)×109/L and 6.935 (5.2, 8.3)×109/L before and after rIL–2 treatment in rIL–2 group, respectively. The difference was not significant (p>0.05). The neutrophils count was 3.075 (2.2, 4.3)×109/L in rIL–2 group after rIL–2 treatment, that was similar with 3.830 (2.8, 4.9)×109/L before rIL–2 treatment (p>0.05). The IL–6 were 7.765 (1.6, 21.7) pg/mL before rIL–2 treatment. After rIL- 2 treatment, the IL–6 decreased to 3.730 (1.5, 7.7) pg/mL. But the decreasing did not get significant difference (p>0.05). There were no obvious difference in hemoglobin (Hb) levels, platelet (PLT) count, alanine aminotransferase (ALT) levels, aspartate aminotransferase (AST) levels, blood urea nitrogen (BUN) levels and serum creatinine (Scr) levels before and after rIL–2 treatment (130.5 [123.0, 148.8] g/L *vs* 128.0 [120.3, 138.8] g/L, 165.5 [132.5, 243.0] ×109/L *vs* 215.5 [117.8, 254.5] ×109/L, 43.0 [30.0, 59.5] U/L *vs* 52.0 [24.8, 67.8] U/L, 29.5 [21.0, 36.8] U/L *vs* 28.0 [19.0, 31.0] U/L, 4.845 [3.5, 7.4] mmol/L *vs* 4.305 [3.6, 5.1] mmol/L, 69.500 [58.7, 78.7] μmol/L *vs* 65.550 [54.3, 73.8]μmol/L, repectively. All p>0.05) (Table 2).

Comparison of laboratory results in non-rIL–2 group patients before and after rIL–2 treatment

There were a clearly visible increasing in lymphocyte count from 0.875 (0.7, 1.3)×109/L to 1.275 (1.0, 1.6)×109/L (p<0.01) and a decreasing in CRP level from 29.930 (7.6, 47.8) mg/L to 4.585 (2.8, 12.1) mg/L (p<0.05) in non rIL–2 group with regular treatment. No obvious effect was demonstrated on change in WBC count (5.070 [4.0, 7.3]×109/L *vs* 6.075 [4.6, 7.7]×109/L, p>0.05), neutrophils count (3.755 [2.5, 6.2]×109/L, 3.925 [2.8, 5.8]×109/L, p>0.05) and serum IL–6 levels (6.070 [1.5, 15.9] pg/mL, 2.445 [1.5, 8.0] pg/mL, p>0.05). In addition, regular treatment had no effect on Hb levels, PLT count, ALT levels, AST levels, BUN levels and Scr levels (123.500 [110.8, 139.8]g/L *vs* 131.000 [102.0, 137.8]g/L, 195.500 [156.5, 270.5]×109/L *vs* 217.000 [177.8, 342.8]×109/L, 25.500 [20.3, 52.3] U/L *vs* 31.500 [20.0, 49.8]U/L, 34.000 [24.5, 44.5] U/L *vs* 25.500 [18.3, 33.8]U/L, 4.480 [3.2, 5.5] mmol/L *vs* 4.405 [3.3, 5.9] mmol/L, 61.150 [52.0, 74.8] μmol/L *vs* 60.600 [53.8, 68.5]μmol/L, repectively. All p>0.05) (Table 2).

Comparison of laboratory results in rIL–2 group and non-rIL–2 group patients after rIL–2 treatment

Compared to non-IL–2 group, rIL–2 group had a significant higher level of lymphocytes, which was 1.935 (1.6, 3.0)×109/L in rIL–2 group vs 1.275 (1.0, 1.6)×109/L in non-rIL–2 group (p<0.01). The level of CRP did not show a significant difference between the two groups (3.965 [1.5, 5.7] mg/L in rIL–2 group vs 4.585 [2.8, 12.1] mg/L in non-rIL–2 group, p>0.05). There were no difference in WBC count, neutrophils count and IL–6 levels between the two groups (6.935 [5.2, 8.3]×109/L *vs* 6.075 [4.6, 7.7]×109/L, p>0.05; 3.075 [2.2,4.3]×109/L *vs* 3.925 [2.8, 5.8]×109/L, p>0.05; 3.730 [1.5, 7.7] pg/mL *vs* 2.445 [1.5, 8.0] pg/mL, p>0.05, respectively). The length of hospital stay between the two groups made no difference (34.00 [20.09□47.91] days *vs* 32.70 [17.07□48.33] days, p>0.05) (Table 2).

Drug associated adverse effects of rIL–2 treatment

After regular treatment such as antiviral drugs, antibiotics, corticosteroids and traditional Chinese medicine, all participants were in normal temperature. In rIL–2 treatment group, 4 (20%) cases of fever associated with rIL–2 were reported with the temperature lowered than 38.5℃ for less than 4 days. The patients complained muscle soreness accompanied with fever. No patients with new fever onset was reported in non-rIL–2 group. The difference was significant (p<0.05). However, rIL–2 treatments did not make obvious change in Hb, PLT count, ALT, AST, BUN and Scr levels (p>0.05) (Table 2).

Discussion

Recent studies have shown that lymphopenia was one of the typical laboratory abnormalities of COVID–19[4–8]. Lymphopenia was observed in 44.4% (12/27) of mild patients and 84.6% (11/13) of severe patients at the onset of COVID–19. The absolute counts of lymphocytes in the peripheral blood of the severe patients was significantly decreased at the time point of disease onset and became even greater on 4–6 days later. From 7 to 15 days after disease onset, the lymphocyte counts gradually increased in the severe patients, and reached a comparable level to that of the mild patients at 16 days after disease onset. Sustained decreases in CD3+ , CD8+ and CD4+ T cell counts were also observed in the severe patients[4]. CD4+ T cells decreased remarkably in patients in the early recovery stage of COVID–19. T and B cell clones were highly expanded during the recovery stage in COVID–19 patients[5]. Increasing data from cohort studies and clinical trials had shown that higher CD4+ T-cell counts were associated with reductions in morbidity and mortality from both AIDS and serious non-AIDS conditions, including cardiovascular disease[9]. All these observations revealed that it might be a prospective therapy for COVID–19 to increase lymphocytes number especially CD4+ T cells.

In previous studies and clinical trials rIL–2 has proven an effective means to enhance the efficacy of antitumor immunotherapy[10]. Several studies demonstrated that rIL–2 showed an immunomodulator effect in virus infections. An *in vivo* study in the immunocompetent BALB/c mouse reported repeated

application of rhIL−2 significantly enhanced the antiviral effect of the low initial number of lymphocytes in several tissues in prophylaxis and also in therapy11. In a prospective cohort study in patients with HIV, CD4+ T-cell counts were observed be increased after rIL−2 treatment12. Low-dose recombinant human IL−2 selectively modulated CD4(+) T cell subsets in patients with systemic lupus erythematosus and markedly reduced the activity of disease13.

In this study, we observed that rIL−2 could increase lymphocytes number in peripheral blood. CRP level showed a remarkable decrease after rIL−2 treatment compared with rIL−2 treatment before, but the difference between rIL−2 group and non rIL−2 group did not reach a significant point (p>0.05). Also the clinical outcome of the length of hospital stay was observed unaffected by rIL-2. Recently it was reported that T cells produced IL−2 promoted survival, proliferation, and differentiation and antibodies production of B cells in COVID−19 patients. Consequently, B cells produce numerous SARS-COV−2- specific antibodies to clear viruses 5,14. Additional supplying of rIL−2 to COVID−19 patients stimulated lymphocyes recovery might have potential therapeutic effect including: First, the rising of lymphocytes might be associated with the changes of T cell subsets especially the rising of CD4+ T cells. Second, rIL−2 might mimick the effect of native IL- 2 and stimulate survival, proliferation, and differentiation and antibodies production of B cells, which accelerated the clearance of virus. Third, rIL−2 might relieve inflammation storm by decreasing the levels of cytokines. The precise mechanisms still required more data to be elucidated. The rIL−2 was tolerated in the severe COVID−19 patients. Only four adverse events were reported in rIL−2 group. The adverse reactions were fever (lower than 38.5℃) and muscle soreness. All adverse events reported were mild, transient and totally relieved after stopping rIL−2 treatment.

Additionally, no abnormal changes in laboratory measurements were clinically significant or considered to be related to rIL−2. For the dose of rIL−2 used in this study was ultra-low (≤1 million IU /day), the adverse reaction of venous thromboembolism detected by ultrasound had not been obtained.

There are some limitations in the present study. It was a retrospective single-center case control study. The hospital was assigned by government to accept mostly severe and critical COVID−19 patients. Only severe COVID−19 patients were included in this study. These might cause some selective bias. The results of this study are limited by the small size of the cohort and the short duration of rIL−2 treatment. Also the effect of different dosage of rIL−2 impacted on different stage of COVID−19 had not been observed.

In conclusion, rIL−2 might be used as a prospective therapy in severe COVID−19 patients for it might potentially improve the recovery of disease by increasing lymphocytes number.

Declarations

The participators/patients reported in our paper consented to participate and/or publish. We thank all participators/patients involved in the study.

Author contribution

PH and MEZ designed study, performed statistical analysis and wrote manuscript. MEZ, QW□BW□SQZ and LK collected data. All authors have approved the final version of this paper.

Acknowledgments

We acknowledge all health-care workers from Beijing, Hainan and Anhui medical teams involved in the diagnosis and treatment of patients in the Union Hospital, Tongji Medical College Huazhong University of Science and Technology.

Sources of funding

None.

Disclosures

None.

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Tables

Due to technical limitations, Tables 1-2 are provided in the Supplementary Files section.

Figures

Figure legends

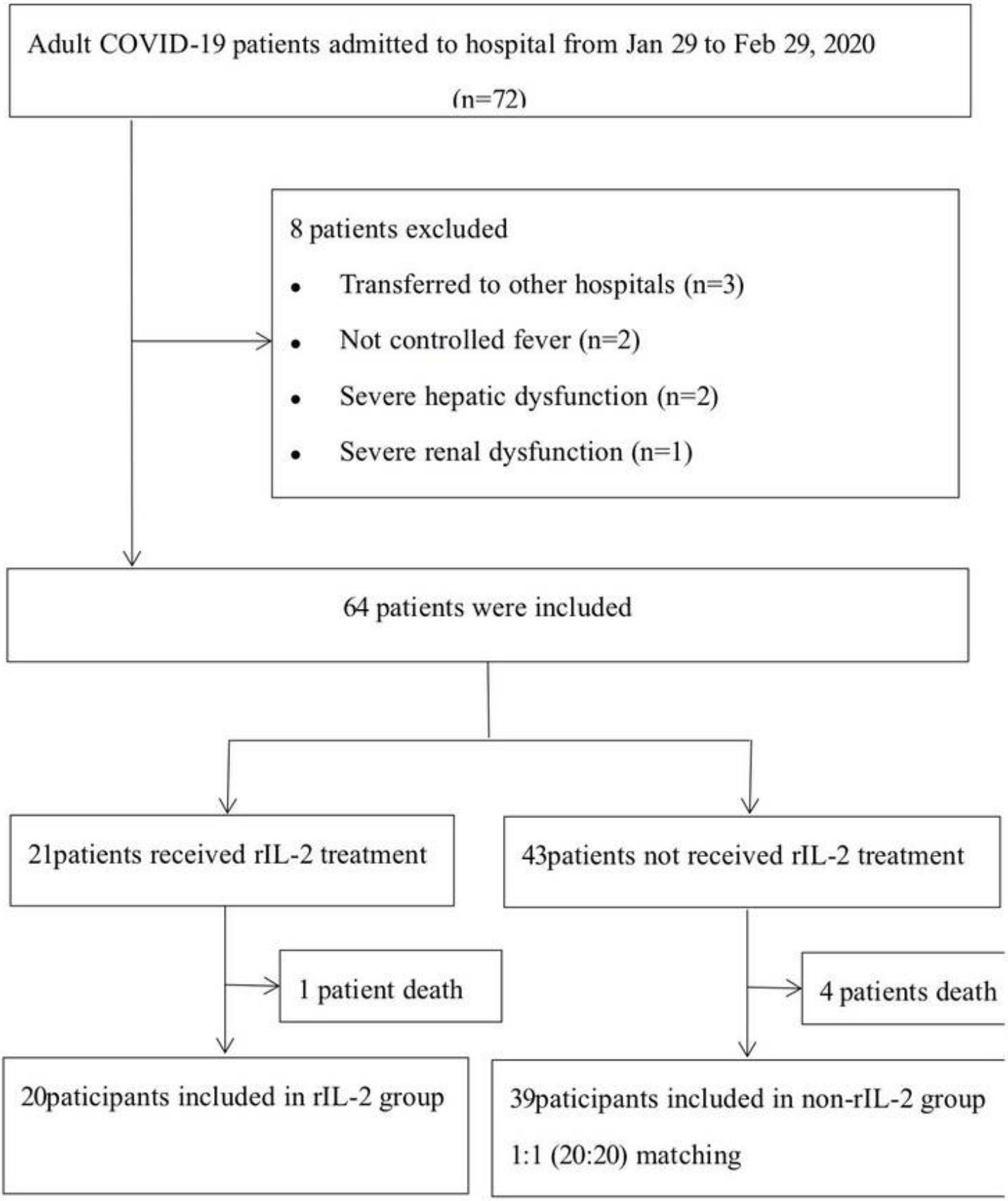

Figure 1. Patient flowchart

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Figure 1

Patient flowchart

Supplementary Files

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