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Restoration of leukomonocyte counts is associated with viral clearance in COVID-19 hospitalized patients

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Summary

Background: Viral clearance is one important indicator for the recovery of SARS-CoV-2 infected patients. Previous studies have pointed out that suboptimal T and B cell responses can delay viral clearance in MERS-CoV and SARS-CoV infected patients. The role of leukomonocytes in viral clearance of COVID-19 patients is not yet well defined.

Methods: From January 26 to February 28, 2020, an observational study was launched at the Department of Infectious Diseases, Zhongnan Hospital of Wuhan University, Wuhan, China. We enrolled 25 laboratory-confirmed COVID-19 patients, whose throat-swab specimens were tested positive for SARS-CoV-2 infection by qRT-PCR. To investigate the factors that contribute to the viral clearance, we comprehensively analyzed clinical records, counts of lymphocyte subsets including CD3+, CD4+, CD8+ T cells, B cells and NK cells in the patients who successfully cleared SARS-CoV-2, and compared to those that failed to, after a standardized treatment of 8-14 days.

Findings: In 25 enrolled COVID-19 patients, lymphopenia was a common feature. After the treatment, 14 out of the 25 enrolled patients were tested negative for SARS-CoV-2. The patients that cleared the infection had restored the numbers of CD3+, CD4+, CD8+ T cells and B

cells as compared to the still viral RNA positive patients, while the recovered patients had a higher count of leukomonocytes.

Conclusions: By comparison of leukomonocytes counts in COVID-19 patients at different stages of the disease, we found that CD3+, CD4+, CD8+ T cells and B cells appear to play important roles in viral clearance. The restoration of leukomonocytes counts from peripheral blood can be used as prognosis for the recovery of an COVID-19 infection. We propose that restoration of leukomonocytes counts can be added to the COVID-19 diagnostic guidance as a criterion for releasing and discharging patients.

Keywords. COVID-19; SARS-CoV-2, leukomonocytes; viral clearance.

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Introduction

In December 2019, a cluster of novel coronavirus-associated pneumonia cases was emerging in Wuhan, China [1]. The disease rapidly spread from Wuhan and caused a wide epidemic in China. The World Health Organization (WHO) has recently named the disease as Coronavirus Disease-2019 (COVID-19) [2]. The etiology of the disease is a novel β-coronavirus, taxonomically named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Until February 29, 2020, SARS-CoV-2 causes an epidemic worldwide. More than 80,000 people in over 60 countries or territories have been contracted of COVID-19, and more than 3000 people have died [3].

The clinical manifestations of COVID-19 cases range from an asymptomatic course to serve acute respiratory symptoms and even acute respiratory failure [4-7]. Transmission occurs from human to human with nosocomial transmissions. The incubation time of COVID-19 has been reported from 0 to 24 days and also asymptomatic carriers can be infectious [8].

As a zoonotic virus, the natural host of SARS-CoV-2 is believed to be bats [9], however, the process of cross-species transmission(s) is still not known. The very first cases were associated with the Huanan Seafood Wholesale Market in Wuhan, China [10]. There is no effective antiviral

drug available yet. Although the pathogenesis of COVID-19 is still unclear, lymphopenia has been found in most COVID-19 patients [10-12]. This feature has also been found in the other two fatal human coronavirus infections caused by severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) [13, 14]. Previous studies showed that T cells, especially CD4+ and CD8+ T cells, play a significant antiviral role in balancing the combat against MERS-CoV or SARS-CoV infections [15]. These results suggested that leukomonocytes counts could be critical indicators associated with severity and the disease outcome. In this study, we retrospectively analyzed 25 COVID-19 cases to elucidate the dynamic of lymphocyte subset changes in the peripheral blood and their roles during the viral clearance.

Methods

Study design

From January 26 to February 28, 2020, 25 COVID-19 patients who had been repeatedly analyzed for lymphocyte subsets and SARS-CoV-2 RNA were enrolled in the study. Informed consents were obtained from all

patients upon admission to the Department of Infectious Diseases, Zhongnan Hospital of Wuhan University, Wuhan, China.

Data collection

The medical records, including epidemiological and demographic information, clinical manifestations, laboratory data, and outcome of disease, were collected. To avoid subjective biases, two investigators reviewed the data independently and made a final consensus.

Peripheral blood (100 μl) was collected from patients at admission to hospital and after 7 – 10 days of standard therapy (Version 6, 2020 Feb 18, http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2.shtml). Lymphocyte subsets such as CD3+, CD4+, CD8+ T cells, B cells and NK cells were stained by using the BD Multitest™ IMK kit (BD Ltd., San Jose, CA, USA) according to the manufacturer´s instruction. The lymphocyte subset analyses were performed by flow cytometry (BD FACSCanto™ II Flow Cytometer).

Serial throat-swab samples were taken from patients at the admission to hospital during 8 – 14 days of standardized treatment (see Table 1) according to the recommendation in the guideline. The swab samples

were placed in 150 μL of virus preservation solution and viral RNA subsequently extracted as described earlier [5, 16]. Two target genes of the SARS-CoV-2 genome, including the open reading frame 1ab (ORF1ab) and the nucleocapsid protein (N) gene, were simultaneously amplified and tested in the real-time RT-PCR assay by using a SARS-CoV-2 nucleic acid detection kit according to the manufacturer's protocol (Shanghai Bio-Germ Medical Technology Co Ltd, Shanghai, China). The reaction mixture consisted of 12 μL reaction buffer, 4 μL of enzyme solution, 3 μL of diethyl pyrocarbonate–treated water, 2 μL of RNA template, and 4 μL of probe/primers solution, which contained two sets of probe primers: one set targets towards to ORF1ab (forward prime 5'-CCCTGTGGGTTTTACACTTAA-3'; reverse primer 5'-ACGATTGTGCATCAGCTGA-3'; and the probe 5'-VIC-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3'), and another targets towards to N (forward primer 5'-GGGGAACTTCTCCTGCTAGAAT-3'; reverse primer 5'-CAGACATTTTGCTCTCAAGCTG-3'; and the probe 5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3').

The results of the real-time RT-PCR analyzes were interpreted as: a cycle threshold value (Ct-value) less than 37 was defined as a positive result; a Ct-value of 40 or more was defined as a negative result; a

158 medium load result, defined as a Ct-value of 37 to less than 40, required
159 another test for confirmation. The patients who repeatedly tested SARS-
160 CoV-2 RNA negative for at least two times with an interval of more than
161 1 day were regarded as viral negative. These diagnostic criteria were
162 based on the recommendation by the National Institute for Viral Disease
163 Control and Prevention, China
164 (http://ivdc.chinacdc.cn/gjhz/jldt/202002/P020200209712430623296.pdf).

165 *Statistical analysis*

166 We used the SPSS 17.0 software package for the statistical analyses. We
167 used X^2 tests or Fisher's exact tests for categorical variables. For
168 measurement of the data, a normal distribution was tested first and then
169 proceeded to t-test, expressed as the mean ± standard deviations;
170 otherwise the Mann-Whitney U test was used for non-normal distribution
171 data, expressed in terms of median (25%–75% interquartile range, IQR).
172 A p value of < 0.05 was considered statistically significant.

173

174 *The principle of medical ethics*

175 This study was approved by the ethics board in Zhongnan Hospital of
176 Wuhan University, Wuhan, China (No.2020011).

177

Results

Baseline Characteristics of COVID-19 patients

A total of 25 patients, 11 men and 14 women were enrolled in this study (Table 1). The ages of the patients spanned from 25 to 80 years (average 51.4±16.6 years). Fourteen people described a previous exposure history to the source of infection, either from the Huanan Seafood Wholesale Market or through direct contacts with COVID-19 patients. The most common symptoms at the onset of illness were: fatigue (80.0%), fever (37.4–39.1^{o}C, 75.0%), myalgia (68.0%), cough (64.0%), and less common: dyspnea (28.0%) and diarrhea (20.0%). Seven cases had underlying diseases such as hypertension, cardiovascular disease, diabetes, malignancy or chronic liver disease (Table 1).

Laboratory analysis before and after treatment

Laboratory analysis were performed before the treatment. The biochemical tests included alanine aminotransferase, aspartate aminotransferase, aspartate aminotransferase, creatinine, and D-dimer, which all were found normal (Table 2). However, blood counts of the patients showed leucopenia (4.5±1.9 $\times 10^{9}$/L) and lymphopenia (< 1.3 $\times 10^{9}$/L). The counts of CD3+ T cells, CD4+ T cells, CD8+ T cells, B cells, and NK cells were found lower than normal values. Interleukin-6

had increased to a mean of 16.1 (3.7 – 31.4) pg/ml. Five patients had an increase of procalcitonin (≥0.05 ng/mL) (Table 2).

The treatment was mainly of supportive care (Table 1). Twenty-four patients were given antiviral therapy including arbidol (orally, 200 mg, three times per day), and oxygen support. Antibiotic therapy, both orally and intravenous, were given as described in Table 1. Fourteen patients received corticosteroids to suppress an excessive inflammatory activation. After standardized treatment of 8–14 days, according to the laboratory analysis of viral RNA, fourteen patients showed viral clearance and 11 patients were still positive for SARS-CoV-2 viral RNA (Table 1).

When comparing the group that showed viral clearance and the group that failed, we found no significant association to sex, epidemiological exposure history, comorbidities, onsets of signs and symptoms, or treatment, but with the age and days of hospitalization. The mean days of hospitalization in the group that showed viral clearance were 12 days (10.0 – 15.0), which was shorter as compared to the group that failed to clear the virus, for which the average days were 21 (18.0 – 23.0) days. Age can play an important role during the viral clearance: patients without viral clearance were older (60.2±16.9, y) than patients with viral

clearance (44.5 ± 13.0 y), showing a statistically significant difference (Table 1).

Association between peripheral lymphocyte counts and disease outcomes

Since lymphopenia was found in all the patients in the current study and also earlier reported elsewhere [10], we further analyzed the association between peripheral lymphocyte counts and disease outcomes. In the 14 patients with viral clearance after the 8 – 14 days of treatment, the counts of CD3+ T cells, CD4+ T cells, CD8+ T cells, and B cells were restored close to normal levels and with a significant difference as compared to the counts at admission in 12 patients. In the remaining 2 patients with viral clearance the leukomonocytes counts were still at low levels (Figure 1, A-E). However, in the patients without viral clearance (No.=11), no differences were seen before and after treatment regarding the CD3+, CD4+, CD8+, B, and NK cell counts (Figure 1, F-J). The association between peripheral lymphocyte counts and viral detection suggested that higher counts of CD3+ T cells, CD4+ T cells, CD8+ T cells, and B cells had a significant impact on the viral clearance.

To further monitor the disease outcome of those 11 patients who were still positive for SARS-CoV-2 virus RNA, we extended the same treatment to 15 – 23 days (Figure 2). At 15 –23 days, 7 patients were negative for viral RNA detection (data for leukomonocyte subsets was available for 6 of these 7 patients), but 4 patients were still positive (all these four had been tested for leukomonocyte subsets). The 4 viral RNA positive patients had low counts of CD3+ T cells (< 1200 $\times 10^9$/L), CD4+ T cells (< 700 $\times 10^9$/L), CD8+ T cells (< 500 $\times 10^9$/L), B cells (< 400 $\times 10^9$/L), and NK cells (< 400 $\times 10^9$/L). Due to the small sample size, no statistically significant differences for the cell counts were observed, except for the NK cell counts, which showed a significant decrease in the 4 viral RNA positive patients (Figure 2, J). However, we found a similar trend that the increased counts of CD3+ T cells and CD8+ T cells corresponded to cases which were negative for viral RNA detection (5 out of 6). One case that still had low counts of CD3+ T cells, CD4+ T cells, CD8+ T cells, B cells and NK cells was found negative for viral RNA.

Clinical outcome

We observed the clinical course of 25 COVID-19 patients within 23 days of treatment. During the first phase of treatment, at 8 – 14 days, fourteen

patients showed viral clearance and 12 of them were discharged from the hospital according to the guideline. The treatment of oxygen support therapy and clinical observation was continued for the remaining 2 patients in this group. Eleven viral RNA positive patients received a second phase of treatment between 15– 23 days. Until February 28, 7 of these 11 patients turned out to be viral RNA negative. Four of these 7 patients were then discharged from the hospital, while the other 3 (one had a liver disease and the other two still had significant clinical symptoms), together with the 4 patients who were still positive for viral RNA, were kept under treatment (Table 1). In total, 9 out of the 25 COVID-19 patients were still hospitalized after 23 days of clinical treatment.

DISCUSSION

In this report, we retrospectively investigated the changes of CD3+, CD4+, CD8+, B, and NK cell counts in peripheral blood of 25 COVID-19 patients during the viral infection and the mechanisms for viral clearance. We found that restoration of CD3+, CD4+, CD8+ T cells, B cells and NK cells was associated the viral clearance in COVID-19 patients.

According to the Guideline of the treatment of COVID-19 (Version 6, 2020 Feb 18, http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2.shtml), the diagnosis of COVID-19 infection is based on an epidemiological history of contact with COVID-19 patients or travel to endemic areas, combined with laboratory testing including viral RNA detection and radiological findings. COVID-19 patients have similarities of the clinical features with other coronavirus infections. Most patients had fever, cough, myalgia and fatigue, and less often showed symptoms of dyspnoea, haemoptysis and diarrhea [10]. Half of the cases had comorbidities such as diabetic, hypertension and cardiovascular diseases [10]. In our study, we also found some cases that had underlying diseases but without any association with the disease outcome. Age is a risk factor for a more severe disease outcome, because of the generally inferior function of the immune system among older people. Based on the guideline, the patients who have normal body temperature for more than three days, mitigation of respiratory symptoms, improvement of radiological evidences for lesions, and had been tested negative for specific SARS-CoV-2 RT-PCR at least twice, can be discharged from the hospital. The average hospitalized time was 17 days (IQR 11.5 – 21.5), and even as long as 23 days for the patients that were released from our study [5], suggesting the time for SARS-CoV-2 clearance is also around

299 17 days or longer. This was also been observed in an earlier study where
300 the shedding of SARS-CoV-2 in saliva continued up to 11 days [17].

301 Until now, there is no effective antivirals available against SARS-CoV-2.
302 To eliminate the virus an effective immunological response with a
303 minimum of immunopathological effects is required [18]. The antigen
304 presentation requires inhibitory alveolar macrophages and dendritic cells
305 (DCs). Successful stimulation can produce viral specific CD4 T cells,
306 which are involved in the development of a specific humoral response,
307 including neutralizing antibodies that can block the viral entry to the cells
308 [19]. CD8+ cytotoxic T cells are required for recognition and killing of
309 the infected cells, further playing a crucial role in SARS-CoV clearance
310 [20, 21]. However, in the biopsy samples from the patients who died of
311 COVID-19, histological examination showed pulmonary oedema with
312 hyaline membrane formation, and interstitial mononuclear inflammatory
313 infiltrates in the lung tissue, suggesting that acute respiratory failure was
314 the cause of death, underlying mechanism that an over-activated
315 immunity injured the lung tissue [12]. Among SARS patients, high
316 expression of dysregulated chemokines and cytokines were found in
317 blood and lungs [22], due to the reason of aberrant immune response
318 caused by the viruses [13, 14]. The delayed or suboptimal immune
319 responses would make chances for SARS-CoV and MERS-CoV to

320 escape from the immune responses, and survive and replicate in the host
321 cells, leading a further delay of the viral clearance [15, 23]. In our study,
322 COVID-19 patients at older ages needed a longer time for the recovery,
323 likely due to the fact that aging is associated with a set of functional and
324 structural alterations in the immune system [24]. Immune-modulators, as
325 corticosteroids, have been empirically used in both SARS and COVID-19
326 patients [10, 25]. Since corticosteroids might have a decline effect of
327 circulating specific B and T cell subsets [26], the usage on COVID-19
328 patients should be carefully evaluated before considered [12, 27].

329 A rapid and generalized lymphopenia has been observed as a prominent
330 part in SARS-CoV and MERS infections [28]. Lymphopenia has also
331 been observed in other viral infections such as measles virus and avian
332 influenza virus, swine foot-and-mouth disease virus, respiratory syncytial
333 virus, and HIV [29-33]. The possible reasons would be lymphocyte
334 sequestration in the lung tissues, or immune-mediate lymphocyte
335 destruction. In MERS infections, the function of bone marrow or thymus
336 suppressed, and furthermore, T cells underwent apoptosis [34-36]. In
337 COVID-19 patients, peripheral CD4 and CD8 T cell counts were found
338 decreased but, they were also hyperactivated, which could result in severe
339 immune injuries [12].

In the present study, we followed the current guidelines and used a specific SARS-CoV-2 realtime RT-PCR for viral detection. Surprisingly, one lymphopenia patient (37y, male, chronic liver disease) was found negative by the realtime RT-PCR and his symptoms had been improved but he is still in hospital. Until now, specific viral RNA detection based on realtime RT-PCR is commonly used in the clinical diagnosis. The usage of realtime RT-PCR is versatile in the detection of SARS-CoV-2 in the respiratory tract specimen or throat swab, but we must keep in mind that those methods always are followed by the probability of false positive/negative. Besides, around 14% of the discharged COVID-19 patients were tested as positive again on follow up samples (https://www.caixinglobal.com/2020-02-26/14-of-recovered-covid-19-patients-in-guangdong-tested-positive-again-101520415.html), showing the recurrence of SARS-CoV-2. This imply that viral detection cannot be used as a single criterion for COVID-19 diagnosis. As a highly contiguous disease with a fatality of approximately 2.3% and up to 49.0% in critical cases [7], every hospitalized COVID-19 patient must be evaluated carefully before discharged. The peripheral leukomonocytes counts could provide more information in the evaluation of COVID-19 patients.

In conclusion, we retrospectively analyzed 25 COVID-19 cases and found that restoration of peripheral leukomonocytes could help the clearance of SARS-CoV-2. We propose here that leukomonocytes counts may serve as a valid prognosis of immune reconstitution for recovery of COVID-19 infection and that can update the current COVID-19 diagnosis guideline.

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476 **Table 1.** Demographics, baseline characteristics, treatment, and clinical

477 outcomes of 25 patients infected with SARS-CoV-2

	Total (n=25)	Viral clearance (n=14)[a]	without viral clearance (n=11)[a]	P value
Sex				
female	14(56.0%)	9(64.3%)	5(45.5%)	0.435
male	11(44.0%)	5(35.7%)	6(54.5%)	
Huanan Seafood Wholesale Market or patient infected with SARS-CoV-2 exposure	14 (56.0%)	9 (64.3%)	5 (45.5%)	0.697
Age, mean ±SD, y	51.4±11.6	44.5±13.0	60.2±16.9	**0.015**
Comorbidities				
Hypertension	3(12.0%)	0(0.0%)	3(25.0%)	0.072
Cardiovascular disease	2(8.0%)	1(7.0%)	1(9.1%)	1.000
Diabetes	2(8.0%)	0(0.0%)	2(18.2%)	0.183
Malignancy	1(4.0%)	0(0.0%)	1(9.1%)	0.440
COPD	0(0.0%)	0(0.0%)	0(0.0%)	-
Chronic liver disease	3(12.0%)	0(0.0%)	3(25.0%)	0.072
Signs and symptoms				
Fever	18 (75.0%)	8 (57.1%)	10 (90.9%)	0.090
Fatigue	20 (80.0%)	11 (78.6%)	9 (81.8%)	1.000
Myalgia	17 (68.0%)	8 (57.1%)	9 (81.8%)	0.234
Cough	16 (64.0%)	9 (64.3%)	5 (45.5%)	0.697
Dyspnea	7 (28.0%)	4 (28.6%)	3 (27.3%)	1.000
Diarrhea	5 (20.0%)	2 (14.3%)	3 (27.3%)	0.623

Headache	2 (8.0%)	0 (0.0%)	2 (13.3%)	0.183
Heart rate, mean ±SD, bpm	88.2±15.1	86.8±11.8	89.9±18.9	0.617
Respiratory rate, median (IQR)	20.0 (19.0, 22.0)	20.0 (19.0, 22.0)	20.0 (19.0, 22.0)	0.845
Mean systolic blood pressure, mean ±SD, mm Hg	130.0±24.4	126.8±22.6	134.2±27.1	0.464
Days from illness onset to hospital, median (IQR), d	6 (3,11)	8 (3,11)	6 (3,11)	1.000
Hospital stays, median (IQR), d	17.0 (11.5, 21.5)	12.0 (10.0,15.0)	21.0 (18.0,23.0)	**0.001**
Treatment				
Oxygen support	15 (60%)	6 (42.9%)	9 (81.8%)	0.099
Antiviral therapy	24(96.0%)	13(92.9%)	11(100.0%)	1.000
Antibiotic therapy	25(100.0%)	14(100.0%)	11(100.0%)	-
Use of corticosteroid	14 (56.0%)	6(42.9%)	8(72.7%)	0.227
Clinical outcome[b]				
Remained in hospital	9(36.0%)	2(14.3%)	7(63.6%)	**0.017**
Discharged	16(64.0%)	12(85.7%)	4(36.4%)	

[a] After 7-14 days, viral clearance or not was determined by viral RNA detections.

[b] After 18-25 days, patients were discharged according to the guideline: 1) a series negative result of viral RNA detection; 2) no clinical symptoms; 3) improved chest CT imaging.

487 **Table 2.** Laboratory results of 25 patients with the outcome of viral

488 clearance.

	Normal Range	Total	Viral clearance(n=14)	without viral clearance (n=11)	P value
White blood cell Count (×10⁹ /L)	3.5-9.5	4.5±1.9	4.5±1.7	4.5±2.2	0.919
Lymphocyte count (×10⁹ /L)	1.1-3.2	**0.9 (0.6, 1.3)**	0.8 (0.6, 1.4)	0.9 (0.6, 1.2)	0.565
Monocyte count (×10⁹ /L)	0.1-0.6	0.37±0.15	0.33±0.15	0.42±0.14	0.117
Neutrophil count (×10⁹ /L)	1.8-6.3	2.8 (1.5, 4.5)	3.1 (1.6, 4.8)	2.6 (1.3, 4.3)	0.805
Platelet count (×10⁹ /L)	125-350	179.5±68.8	191.5±70.4	164.3±66.7	0.336
Alanine aminotransferase (U/L)	9-50	24 (20.5, 38.5)	21 (19, 50.5)	25 (21.0, 35.0)	0.583
Aspartate aminotransferase (U/L)	15-40	26 (21.0, 33.0)	23.0(17.0, 34.8)	28.0(26.0,33.0)	0.066
Total bilirubin (mmol/L)	5-21	9.6 (8.0, 12.7)	9.6 (7.6, 12.2)	10.7 (8.2, 16.7)	0.511
Lactate dehydrogenase (U/L)	125-243	197(164.5, 286.5)	197.0(155.0, 340.0)	201.0(174.0, 279.0)	0.956
Prothrombin time (s)	9.4-12.5	12.3 (11.7, 13.0)	12.6 (11.7,13.5)	12.3 (12.1,12.4)	0.641
Activated partial thromboplastin time (s)	25.1-36.5	29.9±3.0	29.8±2.6	30.0±3.6	0.860
D-dimer, (mg/L)	0-500	187.0 (125.0,309.5)	181.0 (104.5,271.0)	233.0 (145.0,630.0)	0.179
Creatinine (μmol/L)	64-104	65.5±14.6	64.3±14.3	67.0±15.6	0.659
Procalcitonin, ng/mL ≥0.05, No. (%)	<0.05	**5 (20.0%)**	3 (21.4%)	2 (18.2%)	1.000
Interleukin-6 (pg/ml)	0-7	**16.1 (3.7, 31.4)**	13.8 (5.3, 30.2)	17.6 (2.8, 49.9)	0.805
CD3+ T cell count (×10⁹ /L)	805-4459	**561.0 (338.0, 770.0)**	639.0 (400.3, 1038.0)	485.0 (384.0, 732.0)	0.311
CD4+ T cell count (×10⁹ /L)	345-2350	**297.0 (171.5, 447.5)**	327.0 (182.8, 560.5)	220.0 (148.0, 420.0)	0.262
CD8+ T cell count (×10⁹ /L)	345-2350	**272.9±129.7**	291.4±149.3	249.5±101.6	0.435
B cell count (×10⁹ /L)	240-1317	**111.5±75.8**	133.2±84.8	83.8±54.2	0.107
NK cell count (×10⁹ /L)	210-1514	**170.0 (67.5, 245.0)**	193.5 (75.0, 286.0)	170.0 (43.0, 215.0)	0.511

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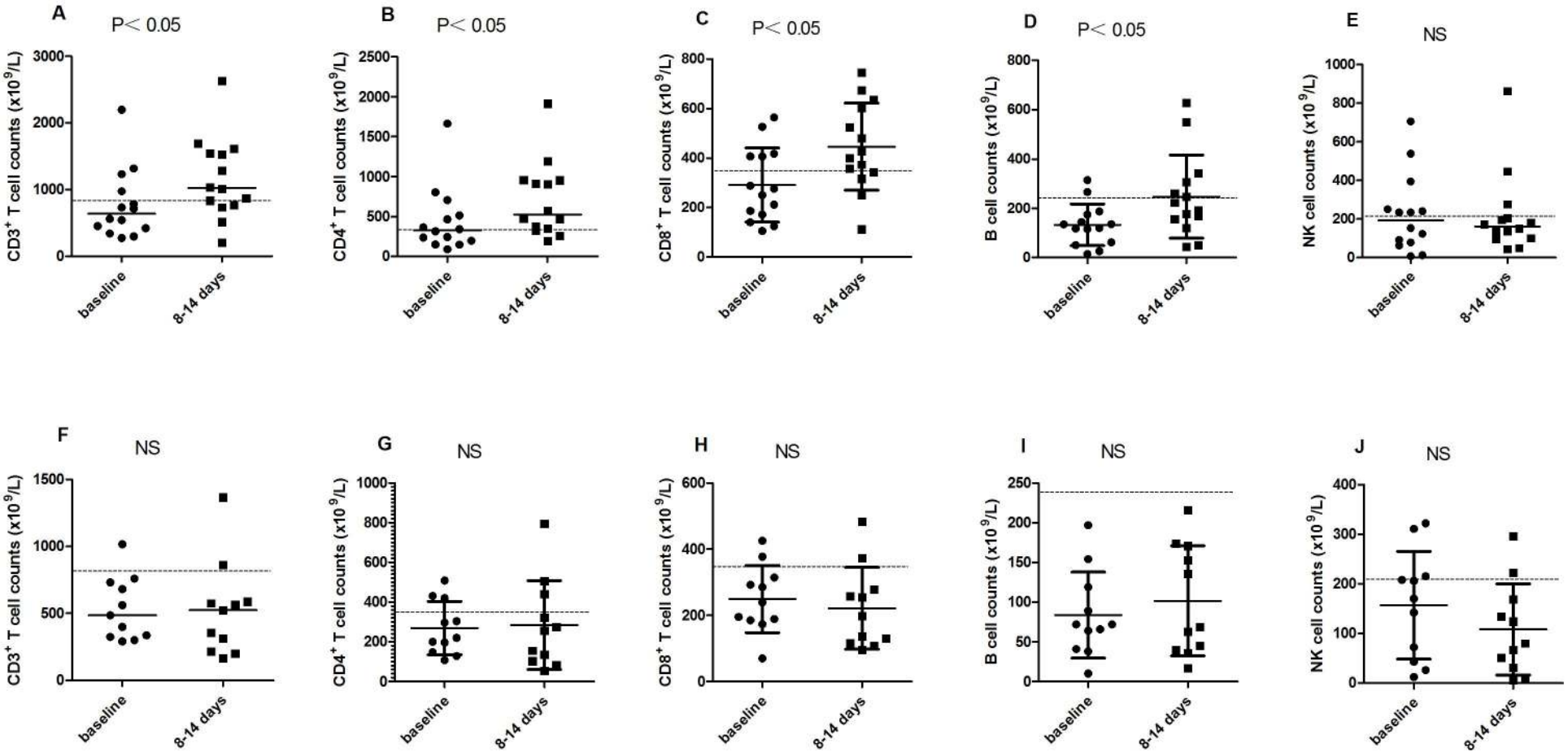
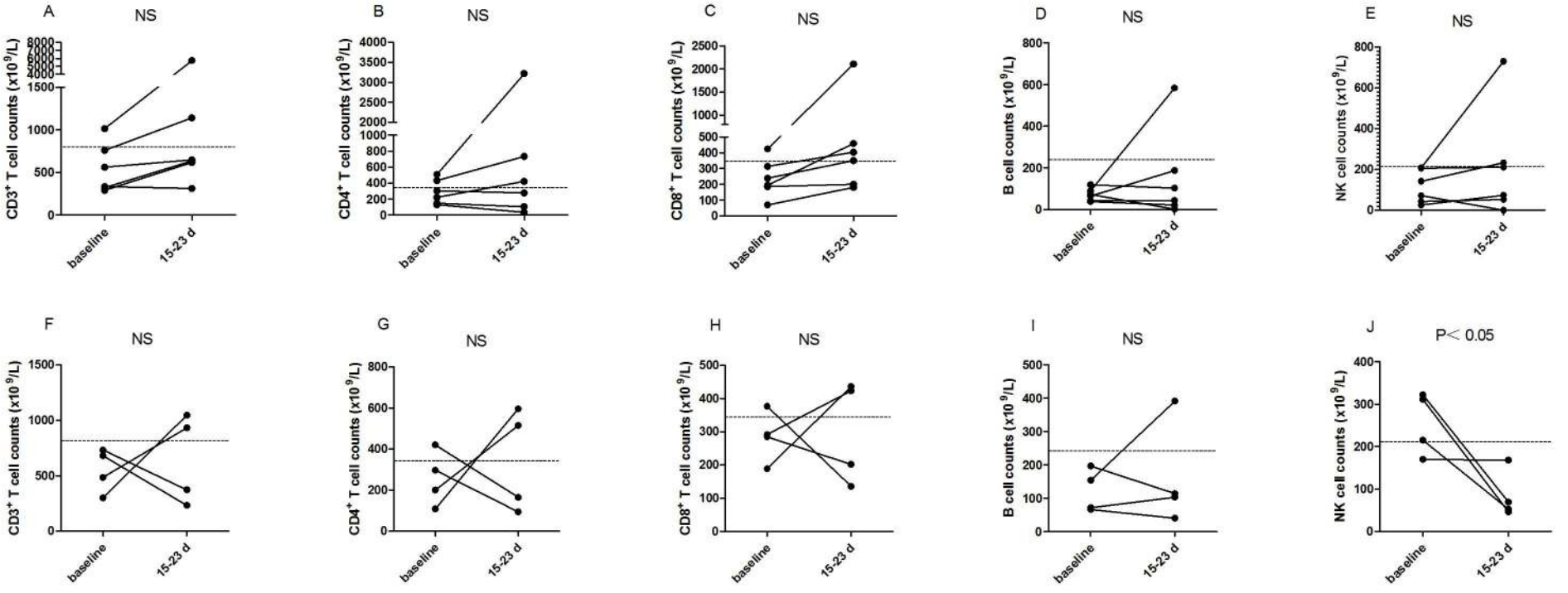
Figure legends

Figure 1. Comparison of peripheral changes in CD3+, CD4+, CD8+ T, B, and NK cell counts in patients who were negative for the SARS-CoV-2 (A-E) and positive (F-J) after 8-14 days of treatment. The medium number of peripheral lymphocyte counts are represented in the solid line. The threshold of normal peripheral lymphocyte counts is represented in the dash line. NS means no significant difference tested in statistical analysis: Mann-Whitney U test was used for A, B, E, F since these data are non-normal distributed and t-test was used for the other normal distributed data).

Figure 2. Comparison of peripheral changes in CD3+, CD4+, CD8+ T, B, and NK cell counts in six patients who were negative for the SARS-CoV-2 (A-E) and four patients who were positive (F-J) after 15-23 days of treatment. The medium number of peripheral lymphocyte counts are represented in the solid line. The threshold of normal peripheral lymphocyte counts is represented in the dash line. NS means no significant difference tested in statistical analysis and all data used Mann-Whitney U test.


A
P< 0.05
CD3+ T cell counts (x10^9/L)
3000
2000
1000
0
baseline
8-14 days
B
P< 0.05
CD4+ T cell counts (x10^9/L)
2500
2000
1500
1000
500
0
baseline
8-14 days
C
P< 0.05
CD8+ T cell counts (x10^9/L)
800
600
400
200
0
baseline
8-14 days
D
P< 0.05
B cell counts (x10^9/L)
800
600
400
200
0
baseline
8-14 days
E
NS
NK cell counts (x10^9/L)
1000
800
600
400
200
0
baseline
8-14 days
F
NS
CD3+ T cell counts (x10^9/L)
1500
1000
500
0
baseline
8-14 days
G
NS
CD4+ T cell counts (x10^9/L)
1000
800
600
400
200
0
baseline
8-14 days
H
NS
CD8+ T cell counts (x10^9/L)
600
400
200
0
baseline
8-14 days
I
NS
B cell counts (x10^9/L)
250
200
150
100
50
0
baseline
8-14 days
J
NS
NK cell counts (x10^9/L)
400
300
200
100
0
baseline
8-14 days


A
NS
CD3+ T cell counts (x10 9/L)
baseline
15-23 d
B
NS
CD4+ T cell counts (x10 9/L)
baseline
15-23 d
C
NS
CD8+ T cell counts (x10 9/L)
baseline
15-23 d
D
NS
B cell counts (x10 9/L)
baseline
15-23 d
E
NS
NK cell counts (x10 9/L)
baseline
15-23 d
F
NS
CD3+ T cell counts (x10 9/L)
baseline
15-23 d
G
NS
CD4+ T cell counts (x10 9/L)
baseline
15-23 d
H
NS
CD8+ T cell counts (x10 9/L)
baseline
15-23 d
I
NS
B cell counts (x10 9/L)
baseline
15-23 d
J
P< 0.05
NK cell counts (x10 9/L)
baseline
15-23 d