Risk factors for prolonged viral clearance in adult patients with COVID-19 in Beijing, China: A prospective observational study

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ABSTRACT

Clearance of COVID-19 from the human body has not been established. Our study collected the laboratory test results from patients and analyzed the correlation between early changes in serum indices and the virus clearance by univariable and multivariable COX regression models, with an aim to explore the risk factors for prolonged viral clearance. The study included 61 patients with COVID-19 treated at the Fifth Medical Center of PLA General Hospital in Beijing from 20 January 2020 to 20 February 2020. We set the total observation of the disease course to 20 days and the patients were divided into two groups (prolonged group, > 20d vs. normal group, ≤ 20d). The 48 patients with COVID-19 included in this study, 13 remained positive for viral nucleic acid monitoring 20 days after onset. The median for virus clearance was 16 days (range, 6–35 days). The results showed that hypertension, a lactate dehydrogenase level > 211.5 U/L, an interleukin 6 (IL-6) level > 12.5 pg/ml, and a NK lymphocyte percentage > 0.5% were associated with prolonged viral clearance. Therefore, we showed that a history of hypertension, an elevated IL-6 level, and an elevated percentage of NK cells were risk factors for prolonged viral clearance.

1. Introduction

In December 2019, novel coronavirus pneumonia (NCP) emerged in Wuhan city, the capital of Hubei and spread rapidly throughout the country. NCP has been defined by the World Health Organization as a global epidemic infectious disease, which was subsequently designated coronavirus disease 2019 (COVID-19) in February 2020 [1,2] As of 13 May, there were 4,347,603 confirmed cases and 294,591 confirmed deaths in 135 countries. COVID-19 appears to have greater infectivity and a lower case fatality rate when compared to SARS and MERS [3,4]. Based on research findings, a consensus in the diagnosis and treatment of COVID-19 was reached in China.

However, clearance of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; previously known as 2019-nCoV) in the human body has not been established. Based on the current data, the duration of SARS-CoV-2 viral shedding may be longer than the other two coronaviruses (SARS-Cov and MERS-Cov). The longest virus clearing time has been reported to be up to 37 days [5]. The duration of infectious virus replication is an important factor for clinicians. There is a significant correlation between the duration of SARS-CoV-2 virus clearance and the prognosis of COVID-19. A longer duration of viral clearance in adult patients with COVID-19 increases the risk of death [6]. Early detection of high-risk patients with prolonged viral clearance is of great importance in guiding the treatment of patients with COVID-19, especially antiviral therapy.

At present, no research has reported an analysis of risk factors for prolonged viral clearance in adult patients with COVID-19. Our study collected the laboratory test results of patients at the time of admission and analyzed the correlation between the early changes in serum indices and the duration of virus clearance, aiming to determine the potential risk factors of prolonged viral clearance.

2. Methods

This study was a single-center prospective observational study, which included patients with COVID-19 treated at the Fifth Medical Center of PLA General Hospital in Beijing from 20 January 2020 to 20 February 2020. All patients were diagnosed with COVID-19 based on positive SARS-CoV-2 RNA PCR results. All patients with a suspected SARS-CoV-2 infection provided respiratory secretion samples at the time of admission. The samples were stored in virus transport medium and transported to Beijing Centers for Disease Control for diagnostic testing.

The epidemiologic history, co-morbidities, vital signs, and symptoms were recorded in detail. Laboratory tests, including a complete blood count, coagulation profile, and serum biochemical panel (renal and liver function, creatine kinase, lactate dehydrogenase, erythrocyte sedimentation rate, and interleukin-6 [IL-6]), were performed immediately upon admission. Because SARS-CoV-2 has the greatest effect on lymphocytes, we also performed lymphocyte subpopulation analysis.

After admission, laboratory indices and imaging were repeated, and signs and symptoms, treatments, and outcomes were recorded. The subtype definition of COVID-19 patients was based on the diagnosis and treatment scheme for COVID-19 (Chinese 5th edition). The degree of COVID-19 was categorized as mild, moderate, or severe. The mild type included patients without pneumonia and mild pneumonia. The moderate type was characterized by dyspnea, respiratory frequency ≥ 30/min, blood oxygen saturation $\leq 93\%$, a PaO_2/FiO_2 ratio < 300, and/or lung infiltrates > 50% within 24–48 h. Patients with the severe type had respiratory failure, septic shock, and/or multiple organ dysfunction/failure.

In previous studies [5–7], it was reported that patients with COVID-19 in whom the duration of virus clearance exceeded 20 days were high-risk and did not survive. Based on our previous clinical experience, < 30% of patients with COVID-19 have a virus clearance time > 20 days. Based on this finding, we set the duration of disease course observations to 20 days. During the analysis, patients were divided into two groups based on the duration of viral clearance (prolonged group > 20 d vs. normal group $\leq$ 20 d). The endpoint of this study was 2 respiratory secretion samples (throat swab or sputum) negative for SARS-CoV-2 RNA obtained at least 24 h apart. Patients were excluded if the follow-up evaluation after discharge were positive for virus testing.

2.1. Statistical analysis

The mean (SD) and median (IQR) were used for continuous variables with and without a normal distribution, while numbers (%) were used for categorical variables. Independent group t-tests were used for comparison of means for continuous variables that were normally distributed. Conversely, the Mann-Whitney *U* test was used for continuous variables that were not normally distributed. Proportions for categorical variables were compared using the $\chi 2$ test or Fisher's exact test

Based on the results of previous research and single factor analysis, we selected 9 possible laboratory test indicators to analyze the diagnostic value. Specifically, receiver operating characteristic (ROC) curve analysis, the area under the curve (AUC), sensitivity, specificity, the positive likelihood ratio (PLR), the negative likelihood ratio (NLR), the positive predictive value (PPV), and the negative predictive value (NPV) were determined to evaluate the ability of the potential laboratory markers to predict efficacy.

The Kaplan–Meier method was used to stratify for normal viral clearance analysis, and the log rank test was applied for comparisons between the prolonged and normal groups. To determine the risk factors associated with prolonged viral clearance, univariable and multivariable COX regression models were used. Results are expressed as hazard ratios (HRs) and 95% confidence intervals (CIs). All computer

programming and statistical analyses were performed using the Statistical Product and Service Solutions (SPSS) 26.0 (IBM Inc., Armonk, NY, USA) and R-Studio 3.6.2. A *p*-value < 0.05 was considered statistically significant.

3. Results

Of the 48 patients with COVID-19 included in this study, 13 remained positive for viral nucleic acid monitoring 20 days after onset. The median time to virus clearance was 16 d (range, 6–35 d). The median age of the two groups was statistically different; the normal group was 42 years of age and the severe group was 61 years of age. Nearly one-half of the patients were Wuhan citizens or visited Wuhan recently. There was no significant difference in the prevalence of diabetes between the two groups, but a greater number of patients in the prolonged group had hypertension than the normal group (53.8% vs. 14.3%, $p = 0.005$). The most common symptoms on admission were fever and cough, followed by sputum production and weakness. The proportion of moderately or severely ill patients was nearly 20% in the two groups, and there was no statistical difference. Among the 87.5% of patients who received oral antiviral therapy, the most commonly used medication was Aluvia/Kaletra (Abbott, Chicago, IL, USA). The use of corticosteroids may delay the clearance of viral nucleic acids. Overall, patients with prolonged viral clearance were older, were in poor pretreatment physical health, and more likely had hypertension. The patient characteristics are listed in Table 1.

3.1. Potential factors associated with prolonged viral clearance

We collected a total of 20 laboratory tests from the enrolled patients. Based on univariable analysis, the percentage of lymphocytes, lactate dehydrogenase level, IL-6 level, prealbumin level, serum ferritin level, erythrocyte sedimentation rate (ESR), percentage of T lymphocytes, percentage of NK lymphocytes, and NK lymphocyte count were associated with the delay of viral clearance. We calculated the AUC, sensitivity, specificity, PLR, NLR, PPV, and NPV of these nine serum markers to predict prolonged viral clearance; the results are listed in Table 2. The increased percentage of NK cells had the highest AUC (0.767) and higher sensitivity and specificity than the other measures. Further, the increase in the NK lymphocyte count had a high predictive value, which suggests that the early changes in NK cells may be related to SARS-CoV-2 viral clearance. Based on ROC curve analysis, IL-6 was shown to be an effective indicator for predicting delayed viral clearance in patients with COVID-19 (Fig. 1). Our research and previous studies [6,7] have shown that among patients with COVID-19, lymphocytes, especially T lymphocytes, were significantly decreased. The predictive value of T lymphocytes for viral clearance duration was greater than lymphocytes (AUC, 0.691 vs. 0.651), which suggests that the analysis of lymphocyte subsets in patients with COVID-19 may have better values. Pre-albumin is an indicator of basic nutritional status and can also predict the clearance time of SARS-CoV-2. Lactate dehydrogenase has high sensitivity, but low specificity (0.846 vs. 0.486) (see Fig. 2).

We performed ROC analysis on the 9 possible indicators, calculated the Youden index, then grouped the indicators in the COX model analysis. Patients were divided into two strata according to the cut-off value of IL-6 (low risk, < 12.5 pg/ml; high risk, ≥ 12.5 pg/ml), percentage of NK lymphocytes (low risk, < 16.5%; high risk, $\geq 16.5\%$), LDH level (low risk, < 211.5 U/L; high risk, ≥ 211.5 U/L), and percentage of T lymphocytes (low risk, $\geq 73.5\%$; high risk, < 73.5%). Kaplan–Meier analysis showed no significant statistical differences in the two groups according to the cut-off values of the percentage of T lymphocytes ($p = 0.15$), but there were significant statistical differences in the other three markers (IL-6, $p = 0.014$; NK%, $p = 0.002$; and LDH, $p = 0.011$).

Based on univariable analysis, the probability of prolonged viral clearance was higher in older patients, patients with hypertension, and

Table 1

Demographics and characteristics of patients infected with COVID-19. Values are numbers (percentages) unless stated otherwise.

	All patients (N = 48)	Prolonged (N = 13)	Normal (N = 35)	p value
Characteristics				
Median (IQR) Age, years	47 (35–63)	61 (55–65)	42 (33–50)	0.000
< 65 years	37 (77.1)	6 (46.2)	31 (88.6)	0.002
≥65 years	11 (22.9)	7 (53.8)	4 (11.4)	
Gender				0.218
Male	29 (60.4)	6 (46.2)	23 (65.7)	
Female	19 (39.6)	7 (53.8)	12 (34.3)	
Exposure history				
Traveling or residence to Wuhan	24 (50)	6 (46.2)	18 (51.4)	0.745
Comorbidity				
Hypertension	12 (25.0)	7 (53.8)	5 (14.3)	0.005
Diabetes	5 (10.4)	2 (15.4)	3 (8.6)	0.492
Other	6 (12.5)	1 (7.7)	5 (14.3)	0.539
Signs and symptoms at disease onset				
Fever (≥37.3 °C)	40 (83.3)	11 (84.6)	29 (82.9)	0.885
Cough	11 (22.9)	2 (15.4)	9 (25.7)	0.449
Sputum	6 (12.5)	2 (15.4)	4 (11.4)	0.713
Dyspnea	3 (6.3)	1 (7.7)	2 (5.7)	0.801
Weakness	6 (12.5)	2 (15.4)	4 (11.4)	0.713
Myalgia	1 (2.1)	1 (7.7)	0 (0.0)	0.097
Diarrhoea	5 (10.4)	0 (0.0)	5 (14.3)	0.150
Disease Type				0.495
Mild	38 (79.2)	10 (76.9)	28 (80.0)	
Moderate	6 (12.5)	1 (7.7)	5 (14.3)	
Severe	4 (8.3)	2 (15.4)	2 (5.7)	
Median (IQR) Time from illness onset to hospital admission, days	5 (2–7)	6 (3–14)	4(2–7)	0.070
Median (IQR) Days from onset of symptoms to positive viral test, days	3 (1–7)	5(3–14)	4(1–7)	0.070
Median (IQR) Days of hospitalization	17(10–24)	25(20–28)	15(7–19)	0.001
Laboratory findings				
Median (IQR) White blood cell count, ×10^9 per L	4.62 (3.70–5.94)	5.26 (3.79–6.71)	4.44 (3.69–5.77)	0.391
Median (IQR)Lymphocyte percentage, %	30.40 (20.58–39.05)	21.7 (15.76–35.55)	31.7 (23.2–41.9)	0.112
< 21.75	1429.2)	7(53.8)	7320.0)	
≥21.75	33(68.8)	6(46.2)	28(80)	
Median (IQR)Haemoglobin, g/L	136.00 (125.75–147.75)	140.0 (126.5–148)	135.20 (123–147)	0.430
Median (IQR)Platelet count, ×10^9 per L	171.00 (151.75–216.25)	185,0 (159.5–220.5)	170 (149–218)	0.523
Median (IQR) Albumin, g/L	40.00 (36.00–43.75)	38.00 (36–41.5)	40 (36–44)	0.305
Mdian (IQR) Prealbumin, g/L	196.00 (138.75–239.0)	147.0 (116.5–228.5)	200 (152–240)	0.182
Median (IQR) Lactate dehydrogenase, U/L	217.50 (181.25–260.75)	2424.0 (210.5–308)	210 (171–262)	0.223
Median (IQR) Creatinine, μmol/L	79.00 (68.00–85.00)	79.0 (69.5–85)	79 (65–86)	0.710
Median (IQR) Serum ferritin,ng/ml	335.7 (110.5–549.58)	384.3 (289.7–570)	310.20 (88–530)	0.241
< 246	19(39.6)	7(53.8)	12(34.3)	
≥246	29(60.4)	6(46.2)	23(65.7)	
Median (IQR) Interleukin 6, pg/ml	11.53 (11.53–21.25)	24.63 (17.9–34.17)	8.9 (5.14–16.4)	0.000
< 12.5	30(60.4)	5(38.5)	25(71.4)	
≥12.5	19(39.6)	8(61.5)	11(28.6)	
Median (IQR) Erythrocyte Sdimentation Rate, mm/60 min	18.5 (10.25–39.00)	28 (16.5–43)	13.00 (8–32)	0.086
Median (IQR) D-Dimer, ng/ml	0.32 (0.24–0.59)	0.33 (0.29–0.52)	0.30 (0.23–0.59)	0.684
Median (IQR) Activated Partial Thromboplastin Time(APTT), S	31.85 (28.05–34.78)	33.00 (29.3–36.45)	31.2 (28–34.7)	0.365
Lymphocyte subset classification				
Median (IQR) Lymphocyte count, /μl	1219 (824.25–1462.0)	1000 (781.5–1616)	1243 (930–1462)	0.318
Median (IQR) T lymphocyte percentage, %	70.00 (56.00–77.00)	66.00 (51.5–71.5)	71 (63–78)	0.043
< 73.5	28(58.3)	10(76.9)	18 (51.4)	
≥73.5	20(41.7)	3 (23.1)	17 (48.6)	
Median (IQR) CD4 lymphocyte percentage, %	37.00 (27.25–45.00)	36.00 (27–44)	37 (32–47)	0.825
Median (IQR) CD8 lymphocyte percentage, %	28.00 (24.00–36.50)	28 (21–30.5)	29 (24–37)	0.201
Median (IQR) B lymphocyte percentage, %	11.00 (8.25–13.75)	10 (8.5–12)	12 (8–15)	0.449

Table 1 (*continued*)

	All patients (N = 48)	Prolonged (N = 13)	Normal (N = 35)	*p* value
Median (IQR) NK lymphocyte percentage, %	16.00	23	12	0.005
	(10.00–25.75)	(18–33.5)	(8–21)	
< 16.5	25(52.1)	1(7.7)	24(68.6)	
≥16.5	23(47.9)	12(92.3)	11(31.4)	
Median (IQR) NK lymphocyte count, /ul	164	232	151	0.018
	(123.25–337.50)	(167.5–470.5)	(121–309)	
< 164.5	24(50)	2(15.4)	22(62.9)	
≥164.5	24(50)	11(84.6)	13(37.1)	
Treatment				
Antibiotics	46(95.8)	13(100)	33(94.3)	0.379
Antiviral therapy				0.394
Aluvia/Kaletra	37(77.1)	9(69.2)	28(80.0)	
Arbidol	5(10.4)	1(7.7)	4(11.4)	
NON	6(12.5%)	3(23.1)	3(8.6)	
Use of corticosteroid	20(41.7%)	9(69.2)	11(31.4)	0.018

patients who used corticosteroids (Table 3). The pre-albumin, LDH, IL-6, and serum ferritin levels, ESR, percentage of NK lymphocytes, and the NK lymphocyte count were also associated with a delay in viral clearance. Considering the total number of deaths (n = 48) in our study and to avoid overfitting in the model, 5 variables were chosen for multivariable analysis on the basis of univariable analysis and previous findings. We found that hypertension, a LDH > 211.5 U/L, an IL-6 > 12.5 pg/ml, and the percentage of NK lymphocytes > 16.5% at the time of admission were associated with prolonged viral clearance (Table 3).

4. Discussion

The current study identified several risk factors for prolonged viral clearance in adults in Beijing who were hospitalized with COVID-19. Age and a history of hypertension may lead to longer virus clearance. With respect to laboratory indicators, elevated levels of LDH, IL-6, and NK cells were shown to be high-risk factors for prolonged clearance of the virus. Among the laboratory indicators, we report for the first time that an increased percentage of NK cells was the best serologic indicator for detection efficacy. In our study, the median virus clearance time was 16 days, which is slightly < the 20-day period previously reported [5]. The proportion of patients enrolled in this study using antiviral drugs was nearly 90%, which was significantly higher than the 21% previously reported [5]. This finding confirms that the application of

antiviral drugs can accelerate the virus clearance time. In previous studies, a higher proportion of patients with severe COVID-19 was reported (62% vs. 16.31) [5,8]. Although there is no direct evidence to confirm the correlation between severity of the disease and elimination of the virus, the high content of the virus in critically ill patients may also be another risk factor that generally leads to prolonged clearance of the virus *in vivo*. Based on the results of current research, patients with COVID-19 are still mainly of the mild type, and antiviral drugs have been widely used [6–8]. We gave a detailed description of the anti-viral drug used by the patients, and there was no statistical difference between the two groups of patients (Table 1). Therefore, the virus clearance time in this study may be closer to the actual real world situation.

Previous studies have reported that older patients are at high risk of death from COVID-19 [5,7–9]. The current study confirmed that increased age was associated with prolonged viral clearance in patients with COVID-19 [5,10]. The age-dependent defects in T- and B-cell function and the excess production of type 2 cytokines could lead to a deficiency in control of viral replication and potentially lead to a delay viral clearance. Hypertension co-morbidity is a major focus of COVID-19 research. Previous studies have shown that the proportion of deaths associated with hypertension in patients with COVID-19 has increased significantly [5,7,9,10]. This finding may be related to changes in the renin-angiotensin system (RAS) in patients with hypertension. The RAS is an important neuroendocrine system that is essential in maintaining

Table 2
Predictive value of the potential factors of Prolonged Viral Clearance.

	AUC (95%CI)	SEN (95%CI)	SPE (95%CI)	PLR (95%CI)	NLR (95%CI)	PPV (95%CI)	NPV (95%CI)
NK lymphocyte percentage	0.767	0.923	0.686	2.937	0.112	0.522	0.960
	(0.637–0.897)	(0.778–1.068)	(0.532–0.840)	(1.757–4.910)	(0.017–0.747)	(0.318–0.726)	(0.883–1.037)
NK lymphocyte count	0.723	0.846	0.629	2.278	0.245	0.458	0.917
	(0.567–0.879)	(0.650–1.043)	(0.468–0.789)	(1.397–3.716)	(0.067–0.898)	(0.259–0.658)	(0.806–1.027)
Interleukin 6	0.712	0.615	0.800	3.077	0.481	0.533	0.848
	(0.545–0.879)	(0.351–0.880)	(0.667–0.933)	(1.397–6.778)	(0.237–0.975)	(0.281–0.786)	(0.726–0.971)
T lymphocyte percentage	0.691	0.571	0.769	2.476	0.557	0.870	0.400
	(0.538–0.844)	(0.407–0.735)	(0.540–0.998)	(0.881–6.958)	(0.343–0.905)	(0.732–1.007)	(0.208–0.592)
Erythrocyte Sedimentation Rate	0.663	0.769	0.600	1.923	0.385	0.417	0.875
	(0.491–0.834)	(0.540–0.998)	(0.438–0.762)	(1.163–3.181)	(0.137–1.076)	(0.219–0.614)	(0.743–1.007)
Lymphocyte Percentage	0.651	0.800	0.538	1.734	0.371	0.824	0.500
	(0.481–0.823)	(0.667–0.933)	(0.267–0.809)	(0.942–3.190)	(0.162–0.854)	(0.695–0.952)	(0.238 = 0.762)
Prealbumin	0.626	0.743	0.615	1.931	0.418	0.839	0.471
	(0.430–0.823)	(0.598–0.888)	(0.351–0.880)	(0.945–3.947)	(0.206–0.849)	(0.708–0.968)	(0.233–0.708)
Lactate dehydrogenase	0.615	0.846	0.486	1.645	0.317	0.379	0.895
	(0.449–0.782)	(0.650–1.042)	(0.320–0.651)	(1.106–2.446)	(0.085–1.185)	(0.203–0.556)	(0.757–1.033)
Serum ferritin	0.605	0.846	0.486	1.645	0.317	0.379	0.895
	(0.438–0.773)	(0.650–1.042)	(0.320–0.651)	(1.106–2.446)	(0.085–1.185)	(0.203–0.556)	(0.757–1.033)

AUC, area under curve; SEN, sensitivity; SPE, specificity; PLR, positive likelihood ratio, NLR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.
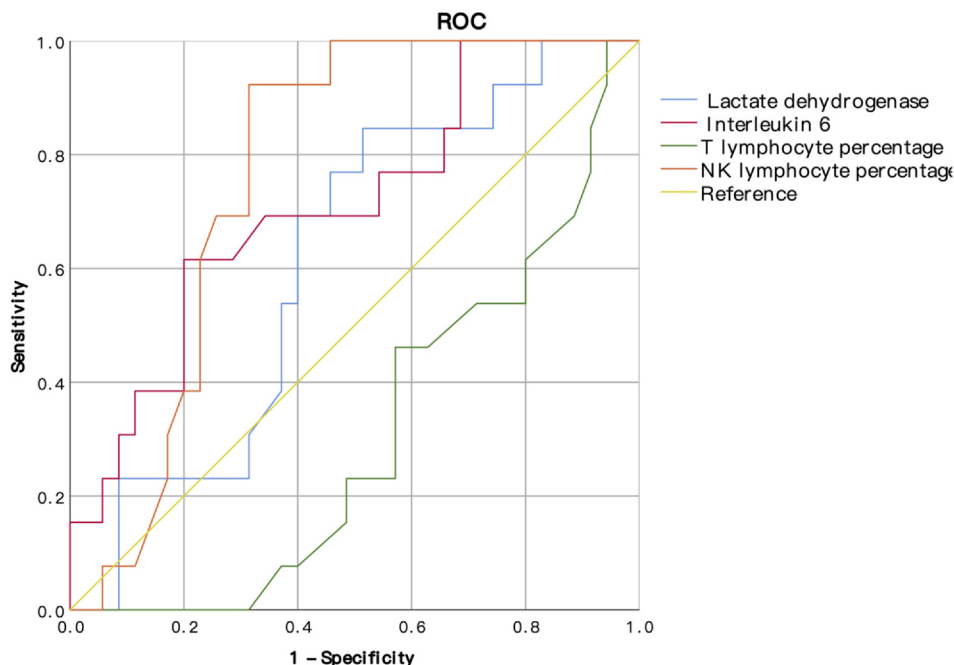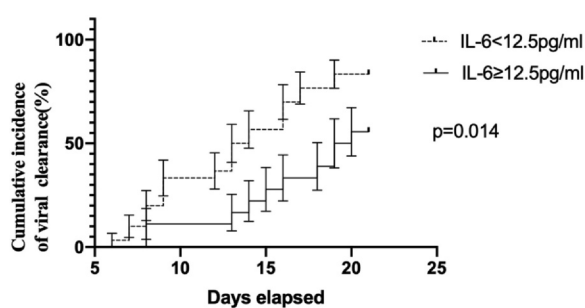
Fig. 1. Receiver operating characteristic (ROC) curve analysis of the potential laboratory marker subsets in predicting the prolonged viral clearance in COVID-19.
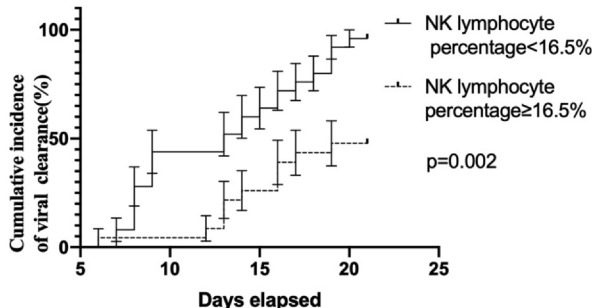
homeostasis. Angiotensin converting enzyme 2 (ACE2) is a key molecule for SARS-CoV-2 infection. The process of SARS-CoV-2 infection in human cells may be affected by binding to the ACE2 molecule. Due to the effects of ACEI/ARB drugs in patients with hypertension, the RAS system is in an abnormal state, which may cause the SARS-CoV-2 to more easily replicate.

IL-6 is an important inflammatory factor with biological activity and

plays an important role in virus clearance. In our study we found that the level of IL-6 in the early stage of COVID-19 was associated with viral clearance. An incremental increase in the IL-6 level is used as a clinical warning indicator of deterioration in COVID-19 [5,6]. Therefore, some researchers suggest that a monoclonal antibody that targets the IL-6 receptor may potentially dampen the immunopathologic changes caused by SARS-CoV-2, and as a result, provide additional time
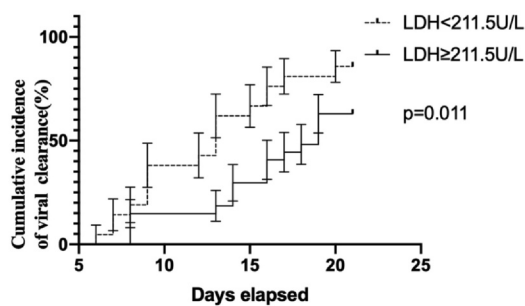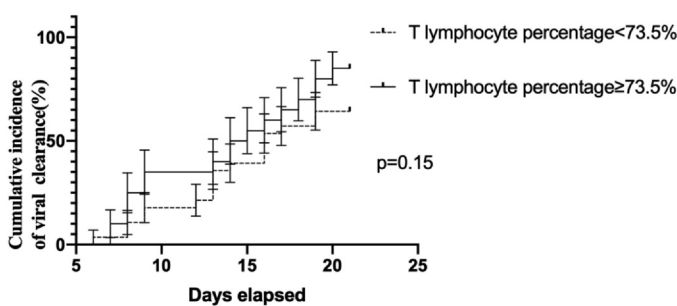

Fig. 2. Kaplan–Meier curves of risk group stratification for normal viral clearance in 2019-nCoV cohorts. (A) Risk group stratification with IL-6, (B) NK lymphocyte percentage, (C) Lactate dehydrogenase and (D) T lymphocyte percent.

Table 3

Cox regression mode using risk factors associated in stratifying prolonged patients.

	Univariable HR (95% CI)	p-value	Multivariable HR (95% CI)	p-value
Age(yr)		0.011		0.057
< 65	1(Ref)		1(Ref)	
≥65	0.259 (0.091–0.738)		0.334 (0.108–1.031)	
Gender		0.138		..
male	1(Ref)			
Female	0.587 (0.291–1.187)		..	
Hypertension		0.009		0.011
NO	1(Ref)		1(Ref)	
YES	0.278 (0.107–0.724)		0.253 (0.082–0.724)	
Diabetes mellitus		0.471		
NO	1(Ref)			..
YES	0.647 (0.198–2.115)		..	
Lymphocyte percentage, %		0.065		..
< 21.74	1(Ref)			
≥21.74	2.109 (0.954–4.660)		..	
Prealbumin, g/L		0.022		..
< 154.5	1(Ref)			
≥154.5	2.444 (1.139–5.244)		..	
Lactate dehydrogenase, U/L		0.018		0.02
< 211.5	1(Ref)		1(Ref)	
≥211.5	0.444 (0.226–0.870)		0.404 (0.189–0.864)	
Interleukin 6, pg/ml		0.022		0.000
< 12.5	1(Ref)		1(Ref)	
≥12.5	0.421 (0.201–0.885)		0.187 (0.078–0.448)	
Erythrocyte Sdimentation Rate, mm/60 min		0.009		
< 18.5	1(Ref)			
≥18.5	0.397 (0.199–0.793)			
Serum ferritin, ng/ml		0.008		..
< 246	1(Ref)			
≥246	0.402 (0.205–0.789)		..	
T lymphocyte percentage, %		0.169		..
< 73.5	1(Ref)			
≥73.5	1.594 (0.820–3.097)		..	
NK lymphocyte percentage, %		0.001		0.000
< 16.5	1(Ref)		1(Ref)	
≥16.5	0.288 (0.139–0.595)		0.182 (0.078–0.424)	
NK lymphocyte count, /μl		0.007		..
< 164.5	1(Ref)			
≥164.5	0.378 (0.188 = 0.762)		..	
Use of corticosteroid		0.015		..
No	1(Ref)			
Yes	0.410 (0.20–0.842)		..	

for virus clearance [11].

In the statistical results of the lymphocyte subsets at the time of admission, we also found that the percentage of NK cells > 16.5% was associated with a prolonged virus clearance time. In our detection system, the normal range of NK cells was 5–27%, and the majority of patients had a gradual decrease with the development of the disease. A previous study reported that the total lymphocyte count, $CD4^+$ T cells,

$CD8^+$ T cells, B cells, and natural killer (NK) cells are decreased in COVID-19 patients, and severe cases had a lower level than mild cases [12]. These alterations were also found in the pneumonia caused by MERS-Cov and SARS-Cov [13]. The number of $CD8^+$ T cells serves as an independent predictor for COVID-19 severity and treatment efficacy. Several studies have highlighted the pivotal role of NK cells in the control of influenza A virus infection in that defects in NK cell activity or depletion of NK cells result in delayed viral clearance and increased morbidity and mortality [14,15]. In our study, we hoped to determine whether the virus clearance time was prolonged by the proportion of NK cells in the early stage of the disease, which also indicates that the severity of the disease is not equal to the virus clearance time.

There were some limitations in the study. First, the study was a single center study with a small sample size. The fairly small sample size reduced the statistical power to detect potential risk factors for prolonged viral clearance. Second, we did not conduct a complete dynamic monitoring of all serum markers during the patient's hospitalization; however, we focused on the early identification of the patients with prolonged viral clearance. Third, we did not discuss the possible effects of drugs and other treatment on viral clereance. As we know, the treatment of COVID-19 is still very controversial, and no drug has been proven to be effective in reducing viral clearance time. Therefore, we did not discuss the effects of drugs in detail.

5. Conclusions

In conclusion, this is the first prospective observational study among patients with COVID-19 with a focus on the viral clearance time. We showed that the history of hypertension, elevated IL-6, and elevated percentage of NK cells at the time of admission were risk factors for prolonged viral clearance in adult patients with COVID-19. We expect that the risk model can help identify high-risk patients with prolonged viral clearance of COVID-19 so that antiviral intervention can be carried out earlier.

CRediT authorship contribution statement

Jian Xue: Data curation, Writing - original draft. **Jing Zheng:** Data curation, Visualization, Investigation. **Xueyi Shang:** Data curation, Visualization, Investigation. **Enqiang Qin:** Supervision. **Peng Zhao:** Supervision. **Yuan He:** Writing - review & editing. **Mengyang Liu:** Software, Validation. **Jin Zhang:** Software, Validation. **Huiying Liu:** Conceptualization, Funding acquisition. **Changqing Bai:** Conceptualization, Funding acquisition.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https:// doi.org/10.1016/j.intimp.2020.107031.

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