Dynamically observing the level of LYM% can Estimate the Time for SARS-CoV-2 Nucleic Acid Test results Turning Negative in COVID-19 Patients

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Abstract

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Abstract: Until now, there are no parameter available for guiding us the proper test scheme for COVID-19 patients during hospitalization so that the physicians will learn their SARS-CoV-2 nucleic acid test results turning negative timely. This study aims to explore feasible parameters to fulfill above requirements. We collected clinical data from 69 patients (31 SARS-CoV-2 positive pneumonia, 38 SARS-CoV-2 negative pneumonia) in Xiangyang Central Hospital (Xiangyang, China) from Feb 12 to Feb 18, 2020 in this study. The general and laboratory data between two groups were compared and discrepant parameters were used to assess the correlation with viral nucleic acid test positive diagnosis. The sensitivity of these parameters to clinical treatment and their correlation with the nucleic acid C_{t} value were also analyzed. WBC, LYM% and PLT decreased, while CRP and Hb increased significantly in SARS-CoV-2 positive patients compared to those in common pneumonia patients. LYM%, Hb and WBC had a good predictive ability to distinguish the nucleic acid positive from negative pneumonia. The dynamics of WBC, LYM%, Hb and CRP in nucleic acid positive patients were more sensitive to clinical treatment and gradually returned to normal level. Only LYM% had a significant correlation with C_{t} value. LYM% dynamics was sensitive to clinical treatment, and significantly correlated with C_{t} value, and might be feasible parameter to estimate the time for SARS-CoV-2 nucleic acid test results turning negative.

Key words: SARS-CoV-2, COVID-19, LYM%, C_{t} value

Introduction

Coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 infection[1], was first identified in Wuhan, China in January 2020 [2-4]. Since then, the number of patients with COVID-19 is dramatically increasing all around the world[5-7].

Nucleic acid testing has played an important role in COVID-19 patient diagnosis, isolation and discharge[8]. However, there is no available parameter can be used to accurately determine when COVID-19 patients will test negative of SARS-CoV-2 after effective treatments until now.

Compared with nucleic acid testing via nasopharyngeal swabs, Blood routine detection is an easier and more convenient method. Dynamic changes in routine blood parameters is not only helpful for COVID-19 diagnosis and typing, but also can discover the changes of patients' condition and the emergence of complications in time, so as to better judge the curative effect and guide the treatment[9].

This study aims to explore feasible laboratory parameter which can be used in clinical practice to estimate the time for SARS-CoV-2 nucleic acid test results turning negative, so as to reduce nucleic acid test frequency to avoid medical resource wastage and lower the infection risk of medical staff.

Material and Methods

Patients

From Feb 12, 2020 to Feb 18, 2020, 69 patients from Xiangyang Central Hospital (31 with SARS-CoV-2-positive Pneumonia and 38 with common Pneumonia) were retrospectively selected. The positive samples were taken from COVID-19 patients diagnosed according to the Diagnosis and Treatment Plan of COVID-19 (7^{th} trial edition) of the National Health, and the negative samples were from the common pneumonia patients with negative nucleic acid diagnosis. All patient or their families signed the informed consent. Subjects meeting the following conditions were excluded from this study: 1) pregnant and lactating women and 2) those with diseases that may affect the efficacy or safety evaluation of this study (hematologic cancer, systemic lupus erythematosus, and rheumatoid arthritis).

Clinical information

The clinical information of all 69 patients we recorded, including hematological and biochemical and C_t value. This protocol was approved by the ethnics committee of Xiangyang Central Hospital, and all procedures were in accordance with the ethnical standards specified by the institution (2020-006).

Quantitative real time polymerase chain reaction (qRT-PCR)

The qRT-PCR detection of viral nucleic acid test (NAT) was one of the most quickly established laboratory diagnosis method in COVID-19 pandemic. After the throat swab specimens in each patient were collected, the presence of SARS-CoV-2 in throat swab specimens was detected by qRT-PCR amplification of SARS-CoV-2 open reading frame 1ab (ORF1ab) and nucleocapsid protein (NP) genes fragments using QIAampRNA kits. C_t value [?]37 was considered positive.

Laboratory test

Venous blood of patients were taken to perform complete blood count and biochemical index, including WBC (White Blood Cell), NE% (Neutrophile%), LYM% (Lymphocyte%), PLT (Platelet), CRP (C-Reactive Protein) and Hb (Hemoglobin).

Statistical analysis

Data are presented as mean ± SD or N (%). Student's t -test andx^2 test was performed to compare the difference between two groups in continuous variables and categorical variables. The differences among three groups were analyzed using one-way ANOVA. ROC (receiver operating characteristic) curves were calculated with AUC (area under curves) estimation for predictive analysis. The Pearson linear correlation analysis was used to estimate the relationship between C_t value and feasible parameters. We considered a P -value less than 0.05 as statistically significant. We used Graph Pad Prism version 5.0 to chart. All statistical analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC) (*$P < 0.05$, ** $P < 0.01$, *** $P< 0.001$).

Results

Comparison of general and laboratory data between two groups

Of the 69 patients with pneumonia included in this study, 31 cases were SARS-CoV-2 positive and 38 were SARS-CoV-2 negative. The indicators between two groups were compared in **table 1** . WBC ($P< 0.001$), LYM% ($P < 0.001$) and PLT ($P< 0.001$) in SARS-CoV-2 positive pneumonia group decreased significantly compared to those in common pneumonia group. In contrast, CRP ($P = 0.027$) and Hb ($P < 0.001$) in SARS-CoV-2 positive pneumonia group increased significantly compared to those in common pneumonia group (**Table 1**). No significant change in NE% was seen between two groups (**Table 1**).

ROC curve for significant different parameters between nucleic acid positive and negative pneumonia group

Factors that were significantly different in above analysis (**Table 1**) were used to analyze the correlation with SARS-CoV-2 nucleic acid positive/negative diagnosis by calculating the AUC (area under curves) of ROC (receiver operating characteristic) (**Figure 1**).

It was apparent that the top three candidates were LYM%, Hb and WBC, whose AUC reached 0.844, 0.757 and 0.712 (**Figure 1**), indicating that they are good predictors to distinguish the SARS-CoV-2 nucleic acid positive from negative pneumonia patients.

Comparison of significant different parameters before, during and after treatment

By comparing the parameters of all patients before, during and after admission, we found that the WBC, LYM%, Hb, CRP of COVID-19 patients were more sensitive to clinical treatment compared with those of common pneumonia patients. The levels of WBC and LYM% increased, while the levels of Hb and CRP decreased with the recovery of COVID-19 patients (**Table 2** , **Figure 2**). CRP level in common pneumonia patients was relatively sensitive to treatment and decreased with clinical treatment(**Table 2** , **Figure 2**).

Spearman's rank correlation coefficient between WBC, LYM%, CRP, Hb and Ct values in patients

The dynamic changes of WBC, LYM%, Hb and CRP in COVID-19 patients were more sensitive to clinical treatment and gradually returned to normal level. The SARS-CoV-2 nucleic acid C_t value will be detected again and again during clinical treatment until their results turned negative for the first time.

Next, we analyzed the correlation between WBC, LYM%, CRP, Hb and C_t values on day 1 and day 7 of admission using spearman method.

The results showed that LYM% dynamic value was significantly correlated with C_t value (P =0.0276 on the first day of admission, P =0.0033 on the seventh day of admission) (**Figure 3**).

Discussion

Our study revealed that WBC, LYM% and PLT decreased, while CRP and Hb increased significantly in nucleic acid positive group compared to those in nucleic acid negative pneumonia group. LYM%, Hb and WBC had a good predictive value to distinguish the nucleic acid positive patients from negative patients. The dynamics of WBC, LYM%, Hb and CRP in nucleic acid positive patients were more sensitive to clinical treatment and gradually returned to normal level. Only LYM% had a significant correlation with C_t value.

COVID-19 caused by SARS-CoV-2 with high transmissibility[10] is an ongoing global pandemic[11]. Given the global spread of COVID-19, strict discharge standard is of great significance for the prevention and control of the epidemic.

At present, the criteria for discharge is 1) temperature returns to normal for more than three days, 2) respiratory symptoms improve significantly, 3) pulmonary imaging shows significant improvement in acute exudative lesions, and 4) nucleic acid test is negative for two consecutive times for samples of sputum, nasopharyngeal swabs, and other respiratory tract specimens, tested at intervals of at least 24 h.

Therefore, negative nucleic acid test is indispensible for patients' discharge[12]. However, there are no available parameters to accurately determine when patients will test negative for SARS-CoV-2 during clinical treatment.

The nucleic acid testing time is based on the clinical improvement of patients which is partially subjective and lacks objective indicators. The clinical and radiographic manifestations of many patients improved significantly with time, but the actual viral load was still high. Therefore, patients often need to take nucleic acid test again and again which brings discomfort to patients during the sampling process, increases the infection risk of medical staff and consumes limited medical resources, resulting in their wastage[13].

Lymphocytes% commonly decreases in the early stage of disease[14, 15] and could be an indicator of reflecting COVID-19 severity and prognosis[16, 17]. LYM% can be used as a reliable indicator to classify the moderate, severe, and critical ill patients independent of any other auxiliary indicators[18]. The COVID-19 is much more serious with lower Lymphocytes [19]. SARS-CoV-2 RNA load is also closely related to COVID-19 severity [20]. The relative RNA load is higher, the lymphocyte count was lower[21].

LYM% can be easily acquired from Blood routine test which requires low cost and the whole process is easier and convenient [22]. There is no standard guideline to accurately determine the timing of SARS-CoV-2 nucleic acid detection until now. Our study confirmed that: 1) LYM% of COVID-19 pneumonia patients was decreased compared to those of common pneumonia patients, 2) LYM% had a good predictive ability to distinguish the nucleic acid positive from negative pneumonia, 3) LYM% dynamics of COVID-19 were sensitive to clinical treatment and 4) LYM% had a significant correlation with C_t value(reverse to viral RNA load). So, we suggested dynamically observing the changes of LYM% may be used to estimate the time for SARS-CoV-2 nucleic acid test results turning negative in COVID-19 Patients.

However, more laboratory test results need to be comprehensively analyzed to explore a quantifiable standard of LYM% to accurately guide the timing of SARS-CoV-2 nucleic acid turning negative detection during the treatment course of COVID-19.

This study has three limitations. First, this is a retrospective study. The cases were only from the Xiangyang

central hospital. Second, only a few relevant studies are available, and this study only analyzed the correlation between LYM% and C_t value, but the quantifiable LYM% is not clear. Third, for greater convenience in statistical analysis, we used a range of lymphocyte count that did not account for the effects of age and gender on the lymphocyte count.

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Table 1. General and laboratory data for SARS-CoV-2 positive and negative pneumonia patients

Items	**SARS-CoV-2-Pos**	**SARS-CoV-2-Neg**	P **value**
Age (years)	51.33 ± 5.53	48.33 ± 4.05	
Gender			
male	17	23	
female	14	15	
WBC(10^*10^9/L)	4.36 ± 0.38 ***	8.98 ± 0.67	$P < 0.001$
NE%(%)	62.10 ± 5.09	64.18 ± 3.80	P= 0.186
LYM%(%)	10.19 ± 1.45 ***	17.93 ± 1.49	$P < 0.001$
PLT(10^*10^9/L)	178.20 ± 19.54 ***	222.50 ± 22.96	$P < 0.001$
CRP (mg/L)	32.74 ± 7.95 *	28.53 ± 7.47	$P = 0.027$
Hb(g/L)	136.0 ± 3.47 ***	110.9 ± 4.71	$P < 0.001$

Table 2. Comparison of laboratory parameters of patients before, during and after treatment

Items	Prior treatment	Prior treatment	During treatment	During treatment	After treatment	After tre
	Neg(-)	**Pos(+)**	Neg(-)	**Pos(+)**	Neg(-)	**Pos(+)**
WBC(10^*10^9/L)	9.0 ±4.2	4.4±1.6	9.7 ± 4.5	7.5±3.9**	9.4±5.8	6.4±1.8*
NE%(%)	64.2±23.4	62.1±22.8	62.6±4.6	67.8±27.7	58.6±24.9	60.4±25
LYM%(%)	20.9±9.6	10.2±8.6	22.2±11.3	16±13.3	23.6±10.6	17.6±15

Items	Prior treatment	Prior treatment	During treatment	During treatment	After treatment	After tre
PLT(10^*10^9/L)	222.5±145.2	178±87.4	266.9±155.6	244±91	304.2±175.6	214±92.
CRP (mg/L)	29.5±28.9	32.7±36.4	10.1±19.1*	9.4±11*	5.5±4.5**	5.0±1.9*
Hb(g/L)	110.9±27.1	136±15.5	109.8±25.9	129.2±13.0	109.9±25.3	124±11.

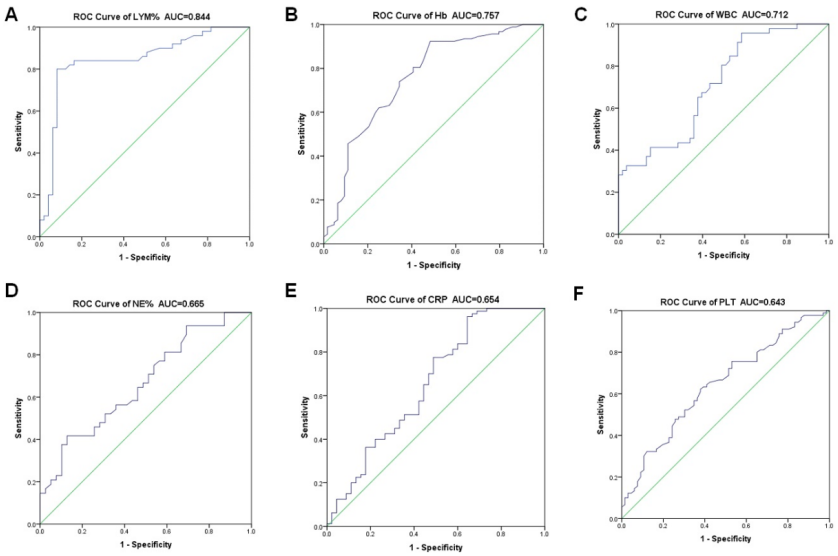

Figure 1. ROC curves of WBC, NE%, LYM%, CRP, PLT and Hb in COVID-19 and common pneumonia groups.

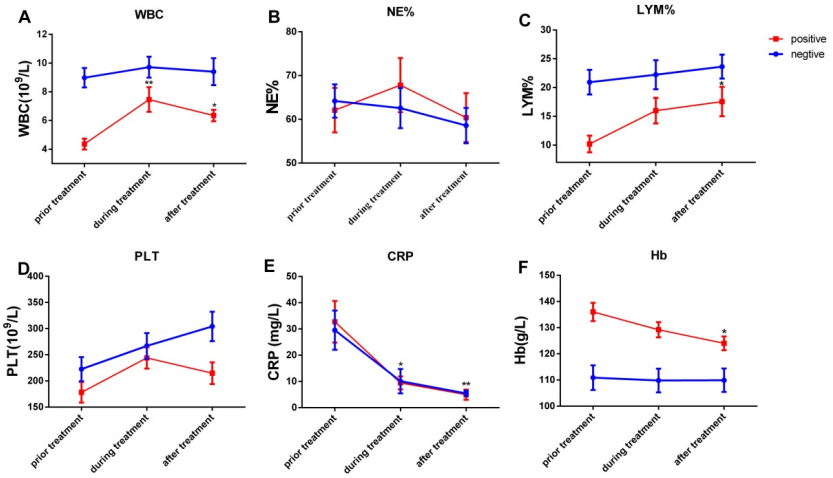

Figure 2. Comparison of laboratory parameters of patients before, during and after treatment.

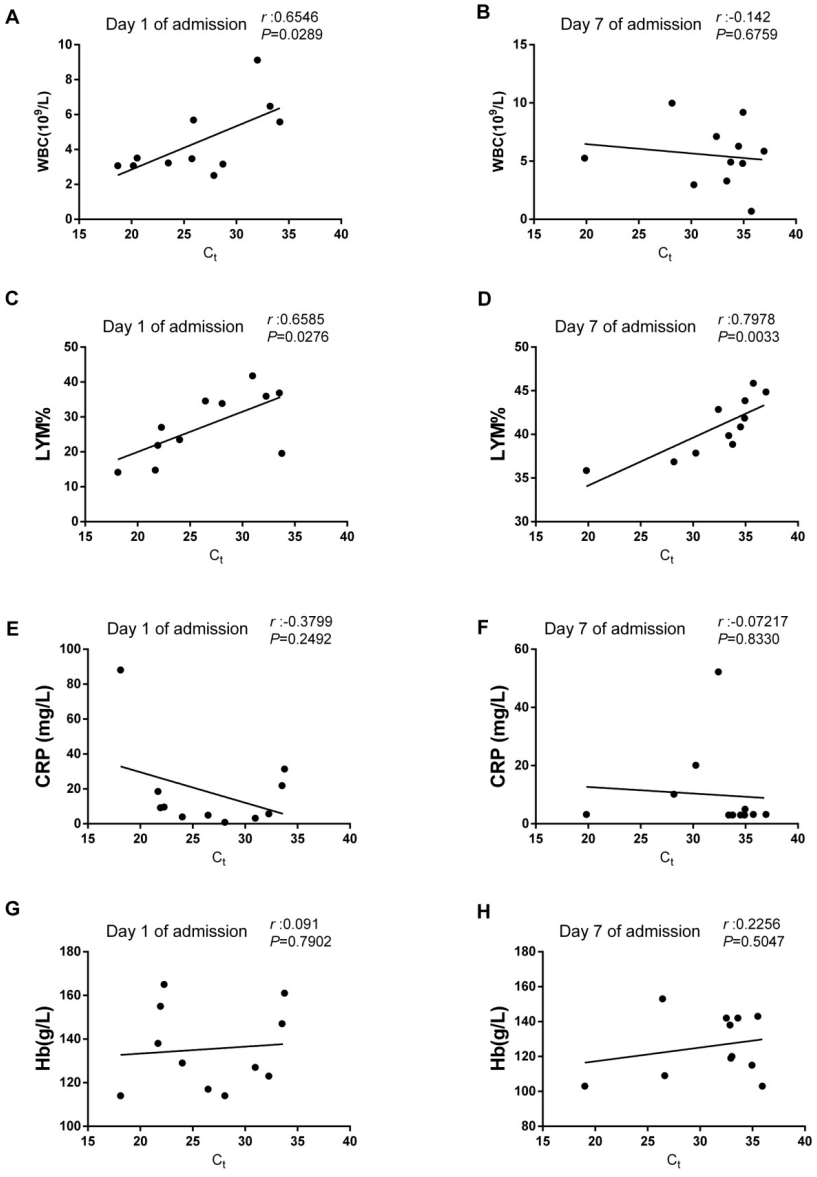

Figure 3. The correlation analysis between C_t value and WBC, LYM%, CRP, Hb in patients. Spearman rank correlation analysis (r) and P values are provided in each graph.