

ORIGINAL PAPER

Laboratory indicators of cytokine storm development in SARS-CoV-2 infection

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Abstract

Objective. This study aims to identify key laboratory indicators associated with the development of cytokine storm in COVID-19 patients, by assessing the predictive value of several blood parameters in a cohort of Romanian patients hospitalized with SARS-CoV-2 infection.

Methods. An analytical observational retrospective study was conducted on 219 COVID-19 patients hospitalized at Colentina Clinical Hospital, Bucharest, from March 1st, 2020, to April 1st, 2021. Patients were categorized into two groups: those who developed a cytokine storm and those who did not, based on clinical and laboratory criteria observed during their hospitalization. Data on demographics, comorbidities, and 13 laboratory parameters were collected. Statistical analysis included univariate and multivariate regression models to determine the impact of these parameters on cytokine storm development.

Results. Of the 219 patients, 68 (31%) developed a cytokine storm. Key parameters such as C-reactive protein (CRP), ferritin, absolute neutrophil count, lactate dehydrogenase (LDH), blood urea nitrogen (BUN) ratio, albumin, lymphocyte percentage and aspartate aminotransferase (AST) were significantly altered in the cytokine storm group, but only CRP, ferritin, absolute neutrophil count, LDH and BUN ratio were significant predictors of cytokine storm progression. No significant associations were found between demographic factors or comorbidities and the risk of cytokine storm development.

Conclusion. Concomitant alteration of CRP, ferritin, neutrophil count, LDH and BUN ratio are effective predictors of cytokine storm in COVID-19 patients. Early identification of these markers variation can aid in clinical decision-making and improve patient management.

Keywords: COVID-19, SARS-CoV-2, cytokine storm, predictive parameters, progression.

Introduction

In late 2019, the first cases of SARS-CoV-2 infection were reported in Wuhan, China. Initially viewed as a localized outbreak, the virus rapidly spread worldwide, prompting the World Health Organization (WHO) to declare a pandemic on March 11, 2020 (with 118,000 cases, 114 affected countries, and 4,291 deaths at that time).

Since then, numerous studies have sought to unravel the intricacies of SARS-CoV-2 infection, known as COVID-19, including its clinical manifestations, complications, and effective therapeutic strategies. Early on, medical teams focused on determining the best treatment protocols. While mild and moderate cases were relatively manageable, severe cases often required aggressive interventions, such as invasive treatments and intensive care, though these efforts sometimes proved futile, resulting in high mortality rates.

A critical factor in severe COVID-19 cases is believed to be a dysregulated immune response, characterized by an excessive release of pro-inflammatory cytokines, often referred to as a cytokine

storm. Similar phenomena are observed in conditions like hemophagocytic lymphohistiocytosis and macrophage activation syndrome, though traditional prediction models for these conditions appear inadequate for COVID-19 [1-3]. To address this, researchers from Temple University developed a predictive model based on laboratory data from 513 hospitalized COVID-19 patients [4]. This study aims to assess the correlation between key laboratory parameters and the onset of cytokine storm in a cohort of approximately 200 Romanian patients hospitalized with SARS-CoV-2, with the goal of enhancing early detection of poor prognosis and thus improving patient outcomes.

Methods

This study is an analytical, observational, retrospective cohort study. The objective is to assess the impact of laboratory parameters on predicting the development of cytokine storms in COVID-19, based on the bloodwork indicators used in the predictive model "Preliminary Predictive Criteria for COVID-19 Cytokine Storm" [4].

For patient selection, we reviewed the electronic

medical records of all patients hospitalized with COVID-19 in the Internal Medicine Ward 3 of Colentina Clinical Hospital in Bucharest from March 1st, 2020 to April 1st, 2021. Initially, a total of 221 patients were selected based on a positive RT-PCR test for SARS-CoV-2 infection, after which the inclusion criteria for the study were evaluated.

The inclusion criteria were defined as follows:

1. Signs and symptoms suggestive of COVID-19 (fever, altered general condition, cough, and dyspnea) at the time of hospital admission or within the last seven days prior to admission;

2. Positive RT-PCR test for SARS-CoV-2 infection;

3. Imaging suggestive of COVID-19 (ground-glass opacities on high-resolution computed tomography [hr-CT] or chest X-ray).

After verifying the inclusion criteria, demographic data (age, sex and comorbidities, including cancer history, pulmonary disease, cardiac disease, hypertension, diabetes, and obesity) were collected from the 221 patients. Laboratory analyses from the entire cohort were gathered, which included parameters used in the baseline study [4]: albumin, lymphocyte percentage, absolute neutrophil count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), fibrinogen degradation products (D-dimers), lactate dehydrogenase (LDH), troponin, anionic gap (AG), chloride, potassium and the BUN: creatinine ratio.

The classification of COVID-19 cases into two categories, with and without cytokine storm, was based on clinical and paraclinical criteria. Patients were considered to have developed cytokine storm if they met the following criteria:

1. Deterioration of respiratory function, defined as an increased oxygen requirement to maintain oxygen saturation above 94%;

2. Elevations of specific markers 3 times above the upper limit of normal (CRP, ferritin, D-dimers, LDH, troponin);

OR

3. Death as a result of SARS-CoV-2 infection.

Following the data collection model utilized by the researchers at Temple University Hospital [4], in order to obtain the most predictive values for each patient's evolution, the laboratory investigations used in this study represented average values from the first seven days of hospitalization for those who did not develop a cytokine storm and up to the day of its development for the others.

After applying the inclusion criteria and excluding patients with incomplete data (2 patients), a total of 219 patients were included in the study, 68 of which developed cytokine storm.

Statistical analysis involved the use of IBM SPSS Statistics, version 20 (SPSS Inc., Chicago, IL, USA). A *p*-value of less than 0.05 was considered statistically significant. Initially, the impact of demographic data on the two patient groups (with and without cytokine storm) was evaluated according to the aforementioned criteria. To establish the influence of dichotomous

variables (sex and comorbidities), the Chi-square test was employed. The impact of age was assessed using the Mann-Whitney U test due to the non-normal distribution (normal distribution was previously tested for all continuous variables using the Shapiro-Wilk test and QQ-plots). Subsequently, a Univariate Binary Logistic Regression was performed to examine the individual impact of each laboratory parameter on the progression to cytokine storm. Then we demonstrated the absence of multicollinearity among the parameters, and then used multivariate analysis with backward selection to identify the most accurate predictive model.

Results

In this retrospective study, 219 patients were included. Among these, a greater proportion were male (59%). The average age was 62.4 years, with the most common comorbidities being hypertension (59%), diabetes mellitus (36%), obesity (20.5%), cardiac disease (31%), pulmonary disease (11%), and neoplasms (12.4%).

Based on the clinical and paraclinical criteria used by physicians to determine which patients were in a cytokine storm, 68 out of the 219 patients (31%) developed cytokine storm.

Higher frequencies of diabetes (44% vs. 32%), obesity (27% vs. 17%), and neoplasms (15% vs. 11%) were observed among patients with a cytokine storm, while the frequency of hypertension was equal in both groups (59%), and pulmonary and cardiac pathologies were more prevalent in those without a cytokine storm (13% vs. 9% and 35% vs. 24%, respectively). Additionally, the average age of patients with a cytokine storm was 62.2 years, comparable to that of the group without a cytokine storm, which was 62.5 years. However, statistical tests revealed no significant association between age, sex, or comorbidities and the risk of developing a cytokine storm (Table 1).

Table 1. Demographic Characteristics According to Cytokine Storm Status.

	%	% without cytokine storm	% with cytokine storm	<i>p</i>
Patient	100	69	31	
Sex				
Women	41	43	38	n.s.
Men	59	57	62	n.s.
Comorbidities				
Cancer	12.5	11	15	n.s.
Pulmonary disease	11	13	9	n.s.
Cardiac Disease	31	35	24	n.s.
Obesity	20.5	17	27	n.s.
Diabetes melitus type II	36	32	44	n.s.
Hypertension	59	59	59	n.s.
Age (years)	62.4	62.5	62.2	n.s.

Table 2 presents the mean values, medians, and standard deviations for two groups: those without cytokine storm and those with cytokine storm. Additionally, the results obtained from binary

univariate logistic regression are included, noting that 8 out of the 13 laboratory analyses exhibited statistically significant changes (increase or decrease, as applicable) in the group with cytokine storm

compared to the group without (specifically: ferritin, PCR, albumin, lymphocyte percentage, neutrophils, AST, LDH, and the BUN ratio).

Table 2. Comparative analysis of laboratory parameters and logistic regression results in patients with and without cytokine storm.

	NV	without CS			with CS			OR (95%CI)*	p
		Mean	Median	Std. Deviation	Mean	Median	Std. Deviation		
Ferritin (ng/ml)	15-150	614.77	520.75	517.26	1375.5	1003	1275.23	1.001 (1.000-1.002)	<0.001
CRP (mg/dl)	0-5	27.82	11.96	36.88	78.07	70.2	70.13	1.019 (1.012-1.027)	<0.001
Albumin (g/dl)	3.4-5.3	3.94	3.99	0.51	3.48	3.7	0.65	0.235 (0.109-0.510)	<0.001
Lymphocyte (%)	20-45	20.66	18.98	8.86	12.56	10.48	9.66	0.888 (0.85-0.92)	<0.001
Neutrophils (*10 ³ /uL)	2-8	6.27	5.16	6.83	9.15	8.64	5.58	1.103 (1.024-1.188)	0.009
GPT (UI/L)	<41	42.51	25	46.1	51.79	37.4	66.28	1.003 (0.998-1.008)	0.25
GOT (UI/L)	<38	32.03	26.05	26.04	69.95	34.6	168.85	1.015 (1.004-1.026)	0.009
D-dimers (µg/mL FEU ³)	0-0.5	0.92	0.51	2.28	1.5	0.84	1.85	1.148 (0.958-1.375)	0.135
LDH (UI/L)	135-225	243.41	219	92.01	486.72	410	303.96	1.014 (1.009,1.018)	<0.001
AG		19.07	20.1	4.29	17.97	18.63	4.81	0.948 (0.084-1.104)	0.49
Clor (mmol/L)	98-110	98.41	100	9.36	99.66	100	5.3	1.028 (0.974-1.086)	0.31
Potassium (mmol/L)	3.6-5.2	4.21	4.21	0.73	4.24	4.21	0.77	1.053 (0.706,1.570)	0.8
BUN:creatinin	6-20	21.18	21.26	11.44	27.37	27.99	8.86	1.058 (1.022,1.095)	1.058

Subsequently, all laboratory parameters were incorporated into a multivariate analysis utilizing a backward selection technique aimed at achieving a predictive model for progression to cytokine storm. Following this analysis, some variables lost their statistical significance, with the majority demonstrating a nonsignificant impact on the model. An exception was ferritin, which, despite not presenting a $p < 0.05$, enhanced the model's accuracy in indicating the progression to cytokine storm. Thus, in alignment with the results of the multivariate analysis, concurrent increases in C-reactive protein, absolute neutrophil count, lactate dehydrogenase, ferritin and BUN ratio were found to most accurately predict the development of cytokine storm (88% of cases).

Discussion

The findings from this study indicate that certain laboratory parameters are statistically significant in distinguishing patients with cytokine storm from those without. Specifically, our analysis identified changes in ferritin, C-reactive protein (CRP), albumin, lymphocyte percentage, absolute neutrophilia, AST, LDH, and the BUN ratio as relevant indicators. Notably, while these parameters demonstrated statistical significance in binary univariate logistic regression, their predictive power for cytokine storm development appears limited, as evidenced by the

multivariate analysis which highlighted only a subset (CRP, neutrophilia, LDH, ferritin, and the BUN ratio) as essential for the predictive model. This aligns with previous findings that indicate elevated ferritin levels, particularly, serve as a biomarker for severe COVID-19 cases (5), reflecting underlying inflammatory processes.

However, our results also raise critical questions about the relevance of individual parameters. For instance, while CRP and LDH have been previously associated with disease severity [6], the odds ratios observed in our study were considerably lower than those reported in the literature (e.g., for CRP - 1.019 vs. 1.341). This suggests a potential contextual variability in these biomarkers' utility, highlighting the need for a comprehensive approach that combines multiple indicators for accurate assessment. The lack of clear cut-off values in our model complicates the clinical application of these findings, as emphasized by the need for standardized thresholds in identifying cytokine storms [2].

Interestingly, we did not find any correlation between demographic factors, such as age or sex, and the development of cytokine storm. This absence of association is somewhat surprising given the established literature indicating that these factors often influence disease outcomes [7]. The implications of this finding warrant further investigation, as demographic factors are typically considered

significant predictors in other studies.

Moreover, the limitations of this study must be acknowledged: the relatively small sample size (n=219) and the fact that laboratory analyses represent averages obtained during days when testing was indicated (not standardized with daily sampling); also, the retrospective design may constrain our findings, considering previous studies have pointed out that a larger cohort would enhance the reliability of predictive models for cytokine storms [7]; additionally, the variability in duration from symptom onset to hospitalization could introduce biases, as delayed presentations may reflect different disease trajectories (8); the absence of an international consensus on the definition of cytokine storm related to COVID-19 adds another layer of complexity, which has been discussed in the literature [9].

Despite these limitations, our predictive model demonstrates considerable potential for identifying patients at risk for severe COVID-19 with progression to cytokine storm. Future models should integrate additional analyses, particularly focusing on pro-inflammatory cytokines such as IL-6, IFN-gamma, and IL-1 β , which have been shown to play significant roles in the pathophysiology of cytokine storms (10). By enhancing our understanding of these biomarkers, we can improve early detection and intervention strategies for patients at high risk.

Conclusion

This study establishes a clear relationship between specific laboratory indicators and the development of cytokine storm in patients with SARS-CoV-2 infection. The identified parameters -ferritin, CRP, absolute neutrophil count, LDH, and BUN ratio - serve as critical markers that can aid in the early detection of severe disease progression. Although demographic

factors such as age and comorbidities did not exhibit significant associations, the prevalence of certain conditions in the cytokine storm group warrants further investigation into their role in immune dysregulation.

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