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Article

Hematological and coagulation parameters as predictors of death by Coronavirus disease in hospitalized patients: a Brazilian follow-up study

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This study aimed to evaluate the hematological and coagulation parameters according to the clinical outcomes of coronavirus disease (COVID-19). We analyzed the hematological and coagulation parameters of hospitalized patients with COVID-19 at admission, and two and three weeks during hospitalization. To assess the performance of these parameters in predicting poor outcomes, receiver operating characteristic (ROC) curves were created. We studied 128 patients with COVID-19 (59.2±17.7 years, 56% male). Non-survivors (n=54, 42%) presented significant alterations in hematological and coagulation parameters at admission, such as increased in white blood cells (WBC), neutrophil, and band cell counts, as well as elevated prothrombin time (PT), activated partial thromboplastin time, and D-dimer levels. During follow-up, the same group presented a gradual increase in D-dimer and PT levels, accompanied by a reduction in PT activity, hemoglobin, and red blood cell count (RBC). ROC curves showed that WBC, neutrophil, and band cell counts presented the best area under the curve (AUC) values with sensitivity and specificity of >70%; however, a logistic regression model combining all the parameters, except for RBC, presented an AUC of 0.89, sensitivity of 84.84%, and specificity of 77.41%. Our study shows that significant alterations in hematological and coagulation tests at admission could be useful predictors of disease severity and mortality in COVID-19.

Keywords: COVID-19. Hematology. Coagulation. Predictors. Death.

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Key Point 1: Alterations in hematological and coagulation data at admission could be useful predictors of disease severity and mortality in patients with COVID-19.

Key Point 2: Hematological and coagulation disorders caused by COVID-19 are associated with death. Identifying these changes at the time of hospitalization affects the treatment and prognosis of patients.

Key Point 3: A multivariate logistic regression model combining several parameters to improve the area under the curve to predict mortality performed better than individual analysis of the parameters.

INTRODUCTION

In December 2019, an outbreak of a novel coronavirus (SARS-CoV-2), which can cause severe acute respiratory syndrome (SARS), occurred in Wuhan (China) and has rapidly infected people worldwide (Wang *et al*., 2020). Thus, the World Health Organization declared the coronavirus disease (COVID-19) a pandemic in March 2020 (Huang *et al*., 2020a). The common symptoms of COVID-19 appear after an incubation period of approximately 5.2 days and are often characterized by flu-like symptoms in addition to anosmia/ageusia and dyspnea (Li *et al*., 2020a). Although the general population is susceptible to SARS-CoV-2 infection, older people have a higher risk of morbidity and mortality, especially those with comorbidities, such as diabetes, hypertension, or cardiac disease (Guan *et al*., 2020).

Early hematological abnormalities in patients with COVID-19 have also been associated with increased mortality risk (Terpos *et al*., 2020). This is probably due to the activation of inflammatory cells, such as neutrophils and monocytes, in addition to endothelial dysfunction, which results in an exacerbated production of procoagulants and uncontrolled cytokine release (Tang *et al*., 2020a). Regarding the parameters that could reflect this state of the exacerbated immune response, studies have been demonstrating that lymphopenia is an effective and reliable indicator of disease severity and the need for hospitalization in patients with COVID-19 (Liu *et al*., 2020a). Moreover, the neutrophil-to-lymphocyte ratio (NLR), which can be easily assessed, has been reported to be a good indicator of a patient's general inflammatory status (Liu *et al*., 2020b).

The American Society of Hematology has outlined that the hematological profile of patients with COVID-19 is associated with a clinical state of hypercoagulation with high D-dimer levels, fibrinogen degradation products, prolonged prothrombin time (PT), and activated partial thromboplastin time (aPTT) (American Society of Hematology, 2020). Thus, it is also important to assess coagulation parameters during the course of COVID-19 because thrombocytopenia is associated with an increased risk of severity and mortality (Lippi, Plebani, Henry, 2020).

Therefore, we designed a longitudinal cohort study to analyze several hematological and coagulation parameters obtained in the clinical routine of hospitalized patients with COVID-19. We aimed to determine whether these parameters could be associated with COVID-19 severity and an increased risk of in-hospital death during the clinical course.

MATERIAL AND METHODS

Study Design

This retrospective study was performed on hospitalized patients with COVID-19 and focused on analyzing hematological and coagulation parameters. Patients included in this study were admitted to the Hospital Universitário Antônio Pedro (HUAP, Niterói, Rio de Janeiro, Brazil) during the initial phase of the COVID-19 pandemic in Brazil from April to August 2020. HUAP is a reference hospital for Metropolitan Region II of Rio de Janeiro State and the current reference treatment center for moderate to severe COVID-19 cases (e.g., persistent cough, fever, and respiratory discomfort or drop in oxygen saturation). Moreover, HUAP is a quaternary hospital, attending to highly complex cases, including cancer, autoimmune disease, heart surgeries, and transplants.

Data were collected at three different time points. The first time point was at admission and confirmation of COVID-19 diagnosis (baseline) and subsequently at the second and third weeks of hospitalization. Patient data (e.g., sex, ethnicity, age, and presence of comorbidities) were obtained from the patients' charts. This study was approved by the Ethics Committee of Universidade Federal Fluminense (CAAE: 30623520.5.0000.5243).

Diagnosis of SARS-CoV-2 Infection

For the molecular diagnosis of SARS-CoV-2 infection, reverse transcriptase real-time polymerase chain reaction (RT-PCR) tests were performed within the first week from the symptom onset. Briefly, viral RNA was isolated from nasopharyngeal swabs or tracheal

aspirates collected from hospitalized patients at admission (within the first week after symptom onset). Importantly, all molecular tests for SARS-CoV-2 diagnosis were performed at the Multiuser Laboratory for Research Support in Nephrology and Medical Science (LAMAP) located at HUAP in accordance with the Brazilian Ministry of Health regulations. The LAMAP has been validated and certified for the diagnosis of SARS-CoV-2 by the Central Public Health Laboratory Noel Nutels (LACEN), a reference laboratory for COVID-19 diagnostics in Brazil.

For viral RNA extraction, the QIAamp Viral RNA kit (QIAGEN, Hilden, Germany) was used according to the manufacturer's instructions. SARS-CoV-2 target gene N1 and N2 amplification and detection by RT-PCR were performed using the 2019-nCOV RUO Kit (catalog number:10006770, Integrated DNA Technologies, Inc., Iowa, USA) and GoTaq® Probe 1-Step RT-qPCR (catalog number: A6121, Promega Corporation, Wisconsin, USA) reagents. Cycle threshold cutoff points were <38 for the N1 and N2 genes and <35 for the internal control (human RNAaseP), following the CDC/USA protocol. Amplification was performed using a 7500 system (Applied Biosystems, Thermo Fisher Scientific, California, USA).

Laboratory Tests

Hematological and coagulation parameters were assessed by automated methods using Coulter LH 750R (Beckman Coulter, California, USA) and Sysmex CA-1500 SystemR (Sysmex America Inc., Illinois, USA), respectively. All tests were performed at the Clinical Pathology Service (HUAP/UFF) within 2–4 h of blood sampling.

We also assessed indirect indicators of the inflammatory state, such as the NLR and monocyte-to-lymphocyte ratio (MLR). The NLR is defined as the absolute number of neutrophils divided by the absolute number of lymphocytes and has been used as an indicator of systemic inflammation (Faria *et al*., 2016). MLR was calculated by dividing the monocyte count by the lymphocyte count obtained from routine blood examination and is a new marker of the systemic inflammatory response that has been investigated in cardiovascular disease (Asan *et al*., 2021; Ramos-Peñafiel *et al*., 2020).

Statistical Analysis

Data are expressed as the mean ± standard deviation (SD) or n (%). Differences between the two groups were assessed using the t-test or Mann-Whitney U test according to the distribution of variables. Paired analysis of longitudinal data was performed using repeated-measures analysis of variance or Friedman test with their respective post-tests. Fisher's exact test was used to calculate the differences between proportions for categorical variables. We performed receiver operating characteristic (ROC) curve analysis for hematological and coagulation parameters obtained at the time of patient admission to investigate their ability to predict the clinical outcome. To investigate the combined ability to predict clinical outcomes, all parameters, except for red blood cell count (RBC), were used in the multivariate logistic regression model. This part of the analysis was performed using the R software (R Foundation for Statistical Computing, Vienna, Austria). Data were analyzed using GraphPad Prism® v.8.0 (GraphPad Inc., California, USA), and statistical significance was set at $P < 0.05$.

RESULTS

In this study, we included 128 hospitalized adult patients with laboratory-confirmed SARS-CoV-2 infection. Overall, the mean age (±SD) of the patients was 59±17 years, and 56% were male. During the follow-up period, 54 patients (42%) died. As expected, the main difference between survivors and non-survivors was older age in the latter group (55±17 vs. 65±16; P=0.0001). Moreover, the number of critical cases (defined as the requirement for invasive mechanical ventilation and hemodynamic instability) was significantly higher in the same group (P<0.0001). The demographic and clinical characteristics of patients with COVID-19 according to clinical outcomes are summarized in Table I.

TABLE I – Clinical and demographic characteristics of hospitalized COVID-19 patients according to outcome from the Hospital Universitário Antônio Pedro (Niteroi, Rio de Janeiro, Brazil)

PARAMETERS	SURVIVORS N = 74	NON-SURVIVORS N = 54	*P*-VALUE
Age, years (mean ± SD)	55.3 ± 17.6	65.8 ± 16.7	**0.0001**
Male gender, n (%)	37 (50)	35 (64.8)	0.1
Symptoms at admission, n (%)			
Fever	56 (75.7)	33 (61.1)	0.08
Cough	51 (68.9)	32 (59.2)	0.3
Sore throat	7 (9.4)	2 (3.7)	0.3
Headache	13 (17.6)	7 (12.9)	0.6
Fatigue	26 (35.1)	13 (24.1)	0.2
Myalgia	11 (14.9)	2 (3.7)	**0.04**
Anosmia/ageusia	11 (14.9)	5 (9.2)	0.4
Diarrhea	9 (12.2)	7 (12.9)	0.9
Dyspnea	37 (50)	34 (62.9)	0.1
Hypoxia (O_2 sat. < 95%)	30 (40.5)	27 (50)	0.4
Comorbidities, n (%)			
Cancer	18 (24.3)	22 (40.7)	0.05
CVD	45 (60.8)	36 (66.7)	0.6
CKD	12 (16.2)	11 (20.4)	0.6
Diabetes	17 (22.9)	23 (42.6)	**0.02**
Obesity	13 (17.6)	13 (24.1)	0.4
Immunosuppression	15 (20.3)	15 (27.8)	0.4
Hematological disease	6 (8.1)	10 (18.5)	0.1
Chronic pulmonary disease	12 (16.2)	11 (20.4)	0.6
Critical cases*, n (%)	9 (12.2)	41 (75.9)	**<0.0001**

Data is presented as n (%) or mean ± SD. (*) Critical cases were defined as requirement of invasive mechanical ventilation and hemodynamic instability. *P*-values were calculated by t-student, Mann- Whitney test or Fisher's exact test (*P*-values < 0.05 are described in bold). CKD = chronic kidney disease; CVD = cardiovascular disease.

First, we performed the analysis of hematological and coagulation parameters of patients with COVID-19 at admission according to the development of clinical outcomes (survivors vs. non-survivors), as shown in Table II. We found that white blood cells (WBC), neutrophil, and band cell counts were significantly increased in non-survivors (P<0.0001). Importantly, lymphocyte and monocyte counts did not show significant differences; however, when we analyzed the percentage of these cells with respect to WBC, both were significantly reduced (P<0.0001 and P=0.0006, respectively). With regard to coagulation parameters, we observed that non-survivors also presented higher PT (P=0.01), aPTT (P=0.0007), INR (P=0.04), and D-dimer (P=0.006) levels than survivors. Of note, no differences were observed in the RBC, hematocrit, hemoglobin, and red cell distribution width (RDW). There was no statistical difference in platelet, lymphocyte, and band cell counts, in addition to RDW percentage, from admission to the subsequent weeks of hospitalization.

TABLE II – Hematological and coagulation parameters at admission, without consider patients with hematological disease

PARAMETERS (mean± SD)	SURVIVORS N = 62	NON-SURVIVORS N = 44	*P*-VALUE
RBC (10^6/mm³)	3.8 ± 0.8	3.6 ± 0.8	0.4
Hematocrit (%)	33.5 ± 7.3	31.8 ± 7.7	0.2
Hemoglobin (mg/dL)	11 ± 2.5	10.4 ± 2.6	0.2
RDW (%)	15.6 ± 2.9	17 ± 4.7	0.1
Platelets (10^3/mm³)	256.1 ± 111.2	249 ± 116.9	0.6
Leukocytes (10^3/mm³)	6.6 ± 3.2	11.5 ± 7.9	**<0.0001**
Neutrophils (10^3/mm³)	4.18 ±	8.56 ±	**<0.0001**
Monocytes (10^3/mm³)	0.47 ± 0.32	0.48 ± 0.39	0.8
Lymphocytes (10^3/mm³)	1.1 ± 0.61	0.96 ± 0.49	0.2
Band cells (10^3/mm³)	0.39 ± 0.39	1.3 ± 1.8	**<0.0001**
Neutrophils (%)	66.8 ± 11.5	73.3 ± 10.2	**0.004**
Monocytes (%)	7.4 ± 4.1	4.7 ± 3.6	**0.0006**
Lymphocytes (%)	18.5 ± 10	10.6 ± 6.6	**<0.0001**
Band cells (%)	5.5 ± 3.6	9.9 ± 6.6	**0.0001**
NLR	5.38 ± 4.25	10.4 ± 7.22	**<0.0001**
MLR	0.47 ± 0.31	0.51 ± 0.36	0.6
PT (sec)	13.9 ± 1.9	15.3 ± 3.2	**0.01**
PT activity (%)	93.7 ± 21.3	82 ± 26	**0.02**
aPTT (sec)	34.4 ± 6.2	40.6 ± 9.8	**0.0007**
INR	1.15 ± 0.57	1.21 ± 0.35	**0.04**
D-dimer (u/mL)	2059 ± 2308	2896 ± 2010	**0.006**

Data is presented as mean ± SD. *P*-values were calculated by unpaired t test or Mann-Whiney test (*P*-values < 0.05 are described in bold). Abbreviations: aPTT = activated partial thromboplastin time; PT = prothrombin time; INR = international normalized ratio; NLR = neutrophil-lymphocyte ratio; MLR = monocyte-lymphocyte ratio; RBC = red blood cells; RDW = red cell distribution width.

When analyzing COVID-19 patients with cancer, the most frequent solid tumor in our cohort were prostate (n=12), lungs (n=5), breast (n=5), brain (n=5), and colon (n=5). We also performed an analysis to investigate the death rate according to the presence of oncological diseases (Table I). We observed a higher death rate among COVID-19 patients with cancer (24.3% vs. 40.7%); however, this result was not statistically significant (P=0.05). Notably, the mortality rate was not associated with any specific type of cancer.

Next, to understand the behavior of blood elements during the progression of COVID-19, we performed a longitudinal analysis of hematological and coagulation parameters at three different time points (baseline, week 2, and week 3). As shown in Table III, we observed a gradual increase in D-dimer (P=0.04) and PT (P=0.02) levels, accompanied by a reduction in PT activity (P=0.03) by

the second week of follow-up. We identified a significant and gradual decrease in hemoglobin levels (P=0.006) and RBC (P=0.008). Furthermore, there was an increase in the WBC count (P=0.008), which was probably due to an increase in the neutrophil count (P=0.04). We also found that the NLR was significantly increased in the non-survivor group at admission (P<0.0001).

Our next step was to perform a longitudinal analysis of hematological and coagulation parameters according to clinical outcomes. As shown in Figure 1, during the course of COVID-19 hospitalization, survivors presented an increase in leukocyte count from baseline to the third week (P<0.05), in addition to a decrease in lymphocyte percentage from the second to the third week (P<0.05). We also observed a gradual increase in D-dimer levels in non-survivors during follow-up, as well as an increase in band cell percentage; however, this increase was not statistically significant in the longitudinal analysis.

TABLE III - Hematological and coagulation parameters according to the hospitalization period regardless of the outcome

PARAMETERS (mean± SD)	BASELINE	WEEK 2	WEEK 3	P-VALUE
RBC (10^6/mm³)	3.7±0.8	3.5±0.9	3.2±0.7	**0.008**[a,c]
Hemoglobin g/dL	10.8±2.5	10.3±2.6	9.3±2.0	**0.006**[a,c]
RDW (%)	16.2±3.8	16.1±3.1	16.3±2.3	0.4
Platelets 10^3/mm³	253.2±113.1	280.9±135.3	298.5±139.4	0.2
Leukocytes (10^3/mm³)	8.6±5.6	10.7±8.4	11.9±2.8	**0.008**[a,c]
Neutrophils (10^3/mm³)	6.2±4.3	7.5±5.8	8.6±6.3	**0.04**[a,c]
Neutrophils (%)	71.1±11.4	70±11.3	73.2±11.4	0.8
Monocytes (10^3/mm³)	0.5±0.3	0.5±0.4	0.7±0.5	**0.02**[a,c]
Monocytes (%)	5.3±3.6	5.2±4	5.7±4	0.8
Lymphocytes (10^3/mm³)	1.0±0.6	1.2±0.7	1.3±0.8	0.2
Lymphocytes (%)	14.15±9.03	13.67±9.99	10.19±7.97	0.2
Band Cells (10^3/mm³)	0.8±1.3	1.1±2.4	1.1±1.2	0.09
NLR	8.40±6.97	9.72±9.88	11.97±8.15	0.2
MLR	0.46±0.34	0.56±0.74	0.73±0.61	0.1
PT (sec)	14.6±2.7	15.7±3.5	16.0±5.5	**0.02**
PT activity (%)	88.3±24.2	78.5±21.8	78.8±21.5	**0.03**
aPTT (sec)	37.2±8.6	40.6±15.8	39.5±13.4	0.8
INR	1.2±0.5	1.2±0.4	1.2±0.3	**0.03**
D-dimer ng/mL	2396±2220	3696±3442	3796±3487	**0.04**

Data is presented as mean ± SD. *P*-values were calculated using Repeated Measures ANOVA or Friedman test with their respective posttests (a, baseline vs. week 2; b, baseline vs. week 3; c, week 2 vs. week 3) (*P*-values < 0.05 are described in bold*)*. Abbreviations: aPTT = activated partial thromboplastin time; PT = prothrombin time; INR = international normalized ratio; NLR = neutrophil-lymphocyte ratio; MLR = monocyte-lymphocyte ratio; RBC = red blood cells; RDW = red cell distribution width.

To analyze the performance of hematological and coagulation parameters measured at admission as predictors of mortality in the context of COVID-19, we analyzed the ROC curves. First, we analyzed each parameter with significant P-values in the bivariate analysis. As shown in Supplementary Table I, we found that WBC (P<0.0001), NLR (P<0.0001), neutrophil count (P<0.0001), and band cell count (P<0.0001) showed the best area under the curve (AUC) values of 0.74, 0.75, 0.77, and 0.79, respectively. In addition, these parameters had sensitivity and specificity values of >70%.

Importantly, the analysis of hematological parameters was performed without considering patients with hematological disease (n=16), as these patients present intrinsic alterations in hematological parameters. The profile of patients with hematological disease is described in Supplementary Table II, and the analysis of laboratory tests at admission in comparison with patients with other comorbidities is reported in Supplementary Table III.

Although the results presented above concerning the analysis of individual parameters as predictors of death in the COVID-19 context were statistically significant, the AUC values were <0.8. To improve these analyses and facilitate the perspective regarding patient prognosis when evaluating hematological and coagulation factors at hospital admission, we performed a multivariate analysis with possible models combining these parameters to predict mortality. For this purpose, we developed a multivariate logistic regression model combining these parameters to improve the AUC, sensitivity, and specificity values. Two different statistical models (A and B) were created to predict mortality. Model A consisted of all hematological and coagulation parameters, and model B consisted of all parameters except the RBC. For model A, we obtained an AUC of 0.88, a sensitivity of 60.6%, and a specificity of 96.7%; for model B, we obtained an AUC of 0.89, a sensitivity of 84.84%, and a specificity of 77.41%. The ROC curves for models A and B are shown in Figure 2. Considering the predictive value for hits and misses, it is important to note that model A indicated that 43 patients survived when 13 of them died, misclassifying 30.2% of cases. Meanwhile, model B indicated that only 5 out of 29 (7.2%) patients survived, thus presenting a lower error rate. Considering that it is more serious to indicate that a patient will survive when, in fact, he dies compared to indicating that he will die when, in fact, he survives, model B performed better. Thus, although model A presented greater specificity, model B was more appropriate because it presented balanced sensitivity and specificity.

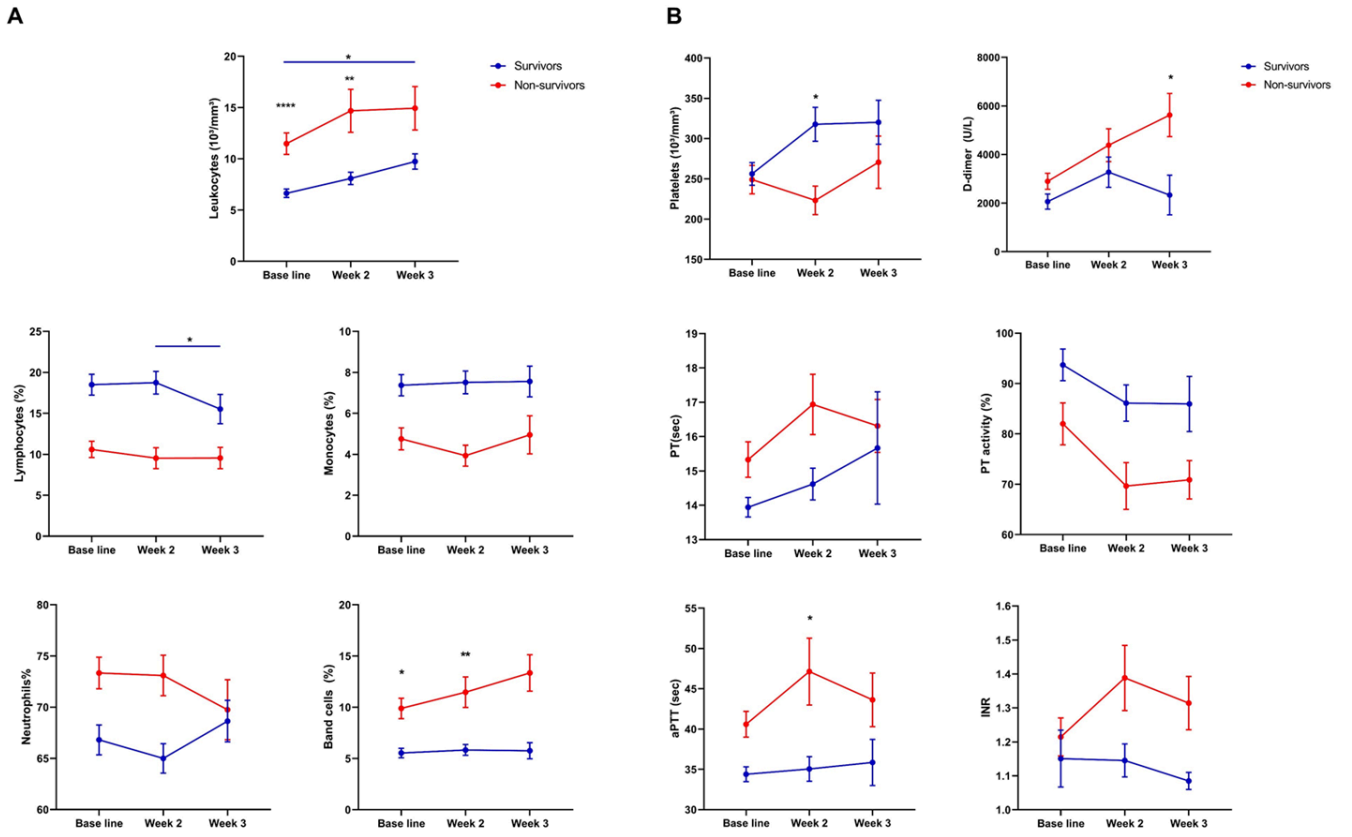

FIGURE 1 - Longitudinal analysis of hematological and coagulation parameters according to the outcome in hospitalized patients with COVID-19 from admission to the third week of hospitalization

"Data is shown as mean ± SEM. (A) Hematological and (B) coagulation parameters according to the outcome (survivors vs. non-survivors). t test or Mann-Whitney tests were used at each timepoint to observe differences between survivors and non-survivors. Repeated Measures ANOVA or Friedman tests were used for the longitudinal analysis, with their respective post-tests (P-values are represented with bars in longitudinal analysis). *P<0.05, **P<0.01 and ***P<0.001. Abbreviations: aPTT = activated partial thromboplastin time; PT = prothrombin time; INR = international normalized ratio."

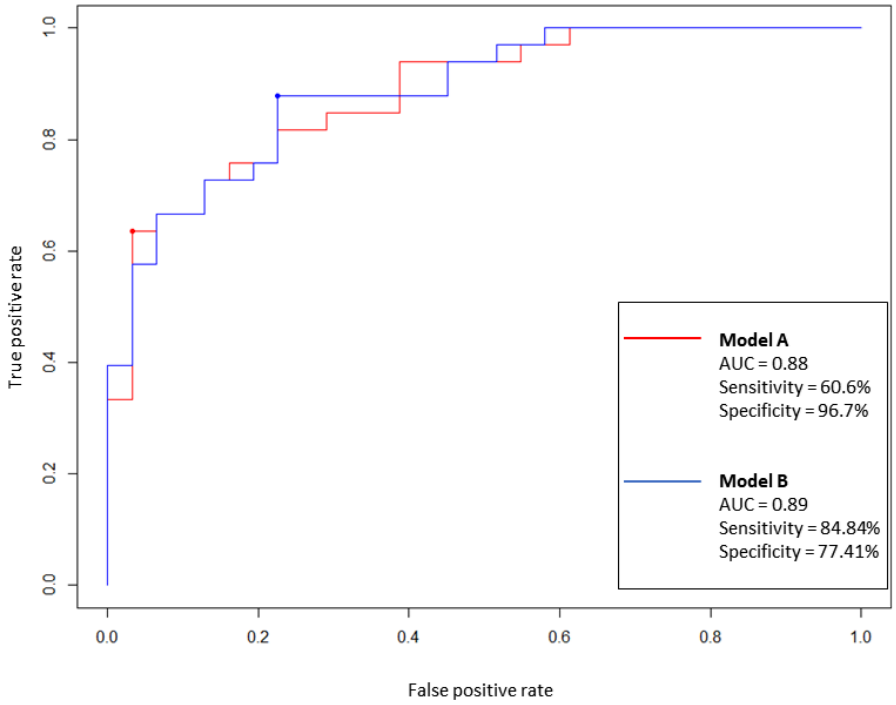

FIGURE 2 - ROC curve provided by the multivariate analysis for predicting death in hospitalized patients with COVID-19
Model A: all hematological and coagulation parameters. Model B: all parameters except for red blood cell count (RBC)

DISCUSSION

In accordance with our results, other studies have reported early hematological abnormalities such as leukocytosis, neutrophilia, lymphopenia, low hemoglobin levels, and platelet counts in critically ill COVID-19 patients (Sun *et al*., 2020; Keski, 2021). According to the literature, although leukopenia and lymphopenia were prevalent in COVID-19 patients in previous studies (Huang *et al*., 2020b; Liu *et al*., 2020b), in our study, we observed lymphopenia associated with leukocytosis. One may suggest that leukocytosis, in this case, could be a reflection of the significant increase in neutrophils and band cells, probably owing to the recruitment of these cells from the bone marrow (Mann *et al*., 2020; Opdenakker, Fibbe, Van Damme, 1998).

In parallel, lymphopenia has been reported in different viral infections, such as those caused by Middle East respiratory syndrome coronavirus (Yang *et al*., 2017) and human respiratory syncytial virus (O'Donnell, Carrington, 2002). In COVID-19, lymphopenia is associated with disease severity and prolonged hospitalization (Liu *et al*., 2020a; Tavakolpour *et al*., 2020).

As a potential biomarker of exacerbated inflammation, the NLR has been investigated in various chronic inflammatory and metabolic diseases, including cardiovascular diseases and oncological processes (Guthrie *et al.,* 2013; Shah *et al*., 2014; Imtiaz *et al*., 2012). As COVID-19 patients who died had neutrophilia and lymphopenia, as mentioned above, this should be expected. In association with an elevated neutrophil count, we also observed a significant increase in band cells in non-survivors of COVID-19. Thus, non-survivor patients are already being admitted to the hospital with high levels of immature WBC, which may contribute to a dysfunctional innate immune response leading to severe lung damage and worse clinical outcomes in patients with COVID-19 (Mann *et al*., 2020).

The presence of neutrophilia in hospitalized patients may be related to COVID-19-associated thrombopathy due to the formation of NETs (extracellular neutrophil traps), which are released by activated neutrophils, stimulating platelet aggregation and triggering the coagulation cascade (Caillon *et al*., 2022; Johnson *et al*., 2022). In addition to the increase in neutrophils, recent data have shown that the SARS-CoV-2 spike protein can stimulate the release of NETs (Youn *et al*., 2021). Moreover, intravascular NET formation with platelet aggregation leads to organ damage, affecting the kidneys, lungs, and heart owing to the rapid occlusion of blood vessels (Leppkes *et al*., 2020; Iliadi *et al*., 2021).

Overall, our results are in accordance with the study performed by Tang *et al.* (2020a, 2020b), which defined a score for disseminated intravascular coagulation risk and observed that D-dimer, PT, and age were positively correlated with mortality in the multivariate analysis (Tang *et al*., 2020a; Tang *et al*., 2020b). We believe that this could reflect an early consumption of coagulation cascade factors since SARS-CoV-2 is likely to promote high fibrin formation and deposition, which can subsequently be associated with higher D-dimer levels, notably in non-survivors (Spiezia *et al*., 2020). This change can also be explained by the exacerbation of inflammation mediated by cytokines and activation of immune cells and is usually accompanied by an increase in the concentration of ferritin and C-reactive protein (Bergamaschi *et al*., 2021).

Other studies have suggested that the NLR may be considered an independent biomarker for predicting disease severity and mortality, indicating poor clinical outcomes (Yang *et al*., 2020; Li *et al*., 2020b). Furthermore, our data showed that aPTT and D-dimer levels were the most relevant coagulation factor parameters. Other studies have shown that PT and D-dimer levels can effectively predict mortality at admission (Liu *et al*., 2020c; Long *et al*., 2020). aPTT values did not correlate with COVID-19 severity in most studies; however, elevated D-dimer levels were strong predictors of both disease severity and mortality (Spieza *et al*., 2020; Huang *et al*., 2020b).

Altogether, hematological and coagulation parameters should be carefully checked at the time of SARS-CoV-2 diagnosis, since early alterations in these routine clinical laboratory examinations could help identify patients at higher risk for death. These data show that abnormalities in these parameters can also be detected as early as at hospital admission in non-survivors of COVID-19.

Our study had some limitations. Patients with hematological diseases were not included in the analysis because they presented alterations in hematological parameters that were associated with the baseline condition. In addition, the presence of different comorbidities could influence the data obtained, as a significant number of patients have cancer, cardiovascular diseases, chronic kidney diseases, chronic lung diseases, diabetes, obesity, and immunosuppression. Nevertheless, several studies have demonstrated that patients with comorbidities constitute the vast majority of severe cases of COVID-19 (Zhou *et al*., 2020; Fathi *et al*., 2021). The disparity between the present study and others may be related to the profile of the patients since it is a reference hospital for critically ill patients with several comorbidities.

CONCLUSION

Our data indicate that COVID-19 patients with moderate or severe disease present significant alterations in hematological and coagulation parameters during the course of hospitalization. Importantly, these alterations were more prominent in non-survivors who presented leukocytosis, neutrophilia, and increased band cells, in association with monocytopenia, lymphopenia, and disturbances in coagulation factors. The analysis of ROC curves showed that, despite the individual analysis of hematological and coagulation parameters presenting statistical significance, the power to predict death by COVID-19 increases when evaluating the parameters altogether. Thus, we hope to contribute to a better understanding of the alterations in routine laboratory tests during hospitalization and draw attention to how these parameters can indicate COVID-19 worsening or even death.

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COMPETING INTEREST

The authors declare no conflict of interest.

REFERENCES

American Society of Hematology. COVID-19 and VTE/Anticoagulation: Frequently Asked Questions, https://www.hematology.org/covid-19/covid-19-and-vte-anticoagulation/; 2020 [accessed 19 December 2022].

Asan A, Üstündağ Y, Koca N, Şimşek A, Sayan HE, Parildar H, et al. Do initial hematologic indices predict the severity of COVID-19 patients? Turk J Med Sci. 2021 Feb 26;51(1):39-44.

Bergamaschi G, Borrelli de Andreis F, Aronico N, Lenti MV, Barteselli C, Merli S, et al. Anemia in patients with Covid-19: pathogenesis and clinical significance. Clin Exp Med. 2021 May;21(2):239-246.

Caillon A, Trimaille A, Favre J, Jesel L, Morel O, Kauffenstein G. Role of neutrophils, platelets, and extracellular vesicles and their interactions in COVID-19-associated thrombopathy. J Thromb Haemost. 2022 Jan;20(1):17-31.

Faria SS, Fernandes PC Jr, Silva MJ, Lima VC, Fontes W, Freitas-Junior R, et al. The neutrophil-to-lymphocyte ratio: a narrative review. Ecancermedicalscience. 2016 Dec 12;10:702.

Fathi M, Vakili K, Sayehmiri F, Mohamadkhani A, Hajiesmaeili M, Rezaei-Tavirani M, et al. The prognostic value of comorbidity for the severity of COVID-19: A systematic review and meta-analysis study. PLoS One. 2021 Feb 16;16(2):e0246190.

Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382(18):1708–20.

Guthrie GJ, Charles KA, Roxburgh CS, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. Crit Rev Oncol Hematol. 2013 Oct;88(1):218-30.

Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020a Feb 15;395(10223):497-506.

Huang I, Pranata R, Lim MA, Oehadian A, Alisjahbana B. C-reactive protein, procalcitonin, D-dimer, and ferritin in severe coronavirus disease-2019: a meta-analysis. Ther Adv Respir Dis. 2020b Jan-Dec;14:1753466620937175.

Iliadi V, Konstantinidou I, Aftzoglou K, Iliadis S, Konstantinidis TG, Tsigalou C. The Emerging Role of Neutrophils in the Pathogenesis of Thrombosis in COVID-19. Int J Mol Sci. 2021 May 20;22(10):5368.

Imtiaz F, Shafique K, Mirza SS, Ayoob Z, Vart P, Rao S. Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. Int Arch Med. 2012 Jan 26;5(1):2.

Johnson JE, McGuone D, Xu ML, Jane-Wit D, Mitchell RN, Libby P, et al. Coronavirus Disease 2019 (COVID-19) Coronary Vascular Thrombosis: Correlation with Neutrophil but Not Endothelial Activation. Am J Pathol. 2022 Jan;192(1):112-120.

Keski H. Hematological and Inflammatory Parameters to Predict the Prognosis in COVID-19. Indian J Hematol Blood Transfus. 2021 Oct;37(4):534-542.

Leppkes M, Knopf J, Naschberger E, Lindemann A, Singh J, Herrmann I, et al. Vascular occlusion by neutrophil extracellular traps in COVID-19. EBioMedicine. 2020 Aug;58:102925.

Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia. N Engl J Med. 2020a;382(13):1199–207.

Li X, Liu C, Mao Z, Xiao M, Wang L, Qi S, et al. Predictive values of neutrophil-to-lymphocyte ratio on disease severity and mortality in COVID-19 patients: a systematic review and meta-analysis. Crit Care. 2020b Nov 16;24(1):647.

Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis. Clin Chim Acta. 2020 Jul;506:145-148.

Liu J, Li H, Luo M, Liu J, Wu L, Lin X, et al. Lymphopenia predicted illness severity and recovery in patients with COVID-19: A single-center, retrospective study. PLoS One. 2020a Nov 18;15(11):e0241659.

Liu Y, Du X, Chen J, Jin Y, Peng L, Wang HHX, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. J Infect. 2020b Jul;81(1):e6-e12.

Liu Y, Gao W, Guo W, Guo Y, Shi M, Dong G, et al. Prominent coagulation disorder is closely related to inflammatory response and could be as a prognostic indicator for ICU patients with COVID-19. J Thromb Thrombolysis. 2020c Nov;50(4):825-832.

Long H, Nie L, Xiang X, Li H, Zhang X, Fu X, et al. D-Dimer and Prothrombin Time Are the Significant Indicators of Severe COVID-19 and Poor Prognosis. Biomed Res Int. 2020 Jun 16;2020:6159720.

Mann ER, Menon M, Knight SB, Konkel JE, Jagger C, Shaw TN, et al. Longitudinal immune profiling reveals key myeloid signatures associated with COVID-19. Sci Immunol. 2020 Sep 17;5(51):eabd6197.

O'Donnell DR, Carrington D. Peripheral blood lymphopenia and neutrophilia in children with severe respiratory syncytial virus disease. Pediatr Pulmonol. 2002 Aug;34(2):128-30

Opdenakker G, Fibbe WE, Van Damme J. The molecular basis of leukocytosis. Immunol Today. 1998 Apr;19(4):182-9.

Ramos-Peñafiel CO, Santos-González B, Flores-López EN, Galván-Flores F, Hernández-Vázquez L, Santoyo-Sánchez A, et al. Usefulness of the neutrophil-to-lymphocyte, monocyte-to-lymphocyte and lymphocyte-to-platelet ratios for the prognosis of COVID-19-associated complications. Gac Med Mex. 2020;156(5):405-411.

Shah N, Parikh V, Patel N, Patel N, Badheka A, Deshmukh A, et al. Neutrophil lymphocyte ratio significantly improves the Framingham risk score in prediction of coronary heart disease mortality: insights from the National Health and Nutrition Examination Survey-III. Int J Cardiol. 2014 Feb 15;171(3):390-7.

Spiezia L, Boscolo A, Poletto F, Cerruti L, Tiberio I, Campello E, et al. COVID-19-Related Severe Hypercoagulability in Patients Admitted to Intensive Care Unit for Acute Respiratory Failure. Thromb Haemost. 2020 Jun;120(6):998-1000.

Sun Y, Dong Y, Wang L, Xie H, Li B, Chang C, et al. Characteristics and prognostic factors of disease severity in patients with COVID-19: The Beijing experience. J Autoimmun. 2020 Aug;112:102473.

Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020a Apr;18(4):844-847.

Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. J Thromb Haemost. 2020b May;18(5):1094-1099.

Tavakolpour S, Rakhshandehroo T, Wei EX, Rashidian M. Lymphopenia during the COVID-19 infection: What it shows and what can be learned. Immunol Lett. 2020 Sep;225:31-32.

Terpos E, Ntanasis-Stathopoulos I, Elalamy I, Kastritis E, Sergentanis TN, Politou M, et al. Hematological findings and complications of COVID-19. Am J Hematol. 2020 Jul;95(7):834-847.

Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet. 2020 Feb 15;395(10223):470-473.

Yang YM, Hsu CY, Lai CC, Yen MF, Wikramaratna PS, Chen HH, et al. Impact of Comorbidity on Fatality Rate of Patients with Middle East Respiratory Syndrome. Sci Rep. 2017 Sep 12;7(1):11307.

Yang AP, Liu JP, Tao WQ, Li HM. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. Int Immunopharmacol. 2020 Jul;84:106504.

Youn YJ, Lee YB, Kim SH, Jin HK, Bae JS, Hong CW. Nucleocapsid and Spike Proteins of SARS-CoV-2 Drive Neutrophil Extracellular Trap Formation. Immune Netw. 2021 Feb 23;21(2):e16.

Zhou Y, Yang Q, Chi J, Dong B, Lv W, Shen L, et al. Comorbidities and the risk of severe or fatal outcomes associated with coronavirus disease 2019: A systematic review and meta-analysis. Int J Infect Dis. 2020 Oct;99:47-56.

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