Neutrophil/ Lymphocyte ratio an important marker of worse outcome in Covid-19: a cross-sectional study in a Brazilian population

Relação neutrófilos/linfócitos um importante marcador de pior desfecho na Covid-19: um estudo transversal em uma população brasileira

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ABSTRACT

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2, is a pandemic with high morbidity and mortality. Several studies on the use of laboratory markers that can differentiate patients at high risk of death associated with COVID-19 or with an unfavourable course from mild to severe disease have been proposed recently. In this study, we evaluated hematological parameters of 1369 patients diagnosed with Covid-19 from a unique center of Brazil and correlated with disease severity. Patients were divided into two groups according to their clinical outcome: death or hospital discharge. The absolute number of neutrophils, lymphocytes, platelets and the neutrophil/lymphocyte ratio were statistically significant between the two groups, with p<0.001. There was no statistical relevance between the platelet/lymphocyte ratio and the clinical evolution of the studied patients. Thus, our results reinforce the data described in previous studies that NLR can be an important marker of unfavourable clinical evolution in Covid -19.

Keywords: Covid-19; lymphopenia; thrombocytopenia; blood plateles; neutrophils; lymphocytes.

RESUMO

A doença por coronavírus 2019 (COVID-19), causada pela síndrome respiratória aguda grave coronavírus 2, é uma pandemia com alta morbidade e mortalidade. Vários estudos sobre o uso de marcadores laboratoriais que podem diferenciar pacientes com alto risco de morte associado à COVID-19 ou com evolução desfavorável de doença leve à grave foram propostos recentemente. Neste estudo, avaliamos parâmetros hematológicos de 1.369 pacientes com diagnóstico de Covid-19 de um único centro do Brasil e correlacionados com a gravidade da doença. Os pacientes foram divididos em dois grupos de acordo com o desfecho clínico: óbito ou alta hospitalar. O número absoluto de neutrófilos, linfócitos, plaquetas e a relação neutrófilos/linfócitos foram estatisticamente significativos entre os dois grupos, com p<0,001. Não houve relevância estatística entre a relação plaquetas/linfócitos e a evolução clínica dos pacientes estudados. Assim, nossos resultados reforçam os dados descritos em estudos anteriores de que a NLR pode ser um importante marcador de evolução clínica desfavorável na Covid -19.

Descritores: Covid-19; linfopenia; trombocitopenia; plaquetas; neutrófilos; linfócitos.

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INTRODUCTION

Coronavírus disease 2019 (COVID-19) is an infectious respiratory disease caused by severe acute respiratory syndrome coronavírus 2 and caused great loss of lives [1]. By April 2022, according to the World Health Organisation (WHO) Covid-19, approximately 6 million people died out of total of > 600 million confirmed cases in all of World. In Brazil, from 3 January 2020 to 1 August 2022, there have been 34.4 million confirmed cases of COVID-19 with 683 thousand deaths, reported to WHO [2]. The Covid-19 pandemic has had repercussions not only of a biomedical and epidemiological nature, but also an impact on health systems, with the exposure of populations and vulnerable groups, the economic support of the financial system and the population.

Covid -19 presents a heterogeneous clinical manifestation from asymptomatic to very serious cases, with a mortality of about 2.4% [3]. Therefore, early identification of patients with a higher risk of poor clinical outcomes and predictive biomarkers are of great relevance.

Several laboratory abnormalities, such as platelets, white blood cell total count, lymphocytes, neutrophils, (together with neutrophil-lymphocyte and platelet-lymphocyte ratio), and hemoglobin were described to be associated with COVID-19 infection and severity. Several studies have shown that these alterations are significantly more common in patients with severe COVID-19 disease and are associated with a poorly prognosis [4-8].

The neutrophil to lymphocyte (NLR) and platelet to lymphocyte (PLR) ratios have been widely used to predict severity of systemic inflammation in a variety of diseases [9-10]. Thus, these correlations appear to be useful markers in the assessment of moderate and severe Covid-19 cases and mortality risk.[4,5]

In this study, we evaluated hematological parameters of 1369 patients diagnosed with Covid-19 and correlated them as NLR and PLR ratios and disease severity.

CASUISTIC AND METHODS

This is a retrospective cross-sectional study with patients with a diagnosis of COVID-19 (SarsCoV-2 infection) admitted from August 2020 to April 2021 at Hospital do Servidor Público do Estado de São Paulo. Whole samples which were confirmed by laboratory test (positive for RT-PCR of nasal and pharyngeal swab) were included. Patients whose medical records were incomplete or who did not have a blood count at the time of admission were excluded from the study. Statistical calculations were carried out based on the total number of patients whose information for that variable was complete. Thus, the total number of patients may vary according to the variable studied. The following clinical information and laboratory data were extracted from medical records. Patients were divided into two groups according to their clinical course: Group 1- death and Group 2- hospital discharge. Their clinical, laboratory data, NLR and PLR ratios were compared.

Continuous and categorical variables were expressed as medians (interquartile ranges) and absolute and relative frequencies, respectively. Comparisons between the two groups (outcome of death or hospital discharge) were performed using chi-square or Mann-Whitney test. We used the IBM SPSS Statistics software package version 24 for statistical analysis. Significance was set at $p<0.05$.

A binary logistic regression was used to examine factors associated with mortality. A hierarchical method was performed with the variables. Multicollinearity was assessed by

examining the variance inflation factor (values > 2 were excluded). The results are presented as odds ratios and 95% confidence intervals.

The Ethics Committee of the healthcare facility approved the study protocol (CAAE: 58792822.1.0000.5463).

RESULTS

A total of 1369 patients aged 59 to 77 years old with COVID-19 were included. Seven hundred and one (51,2%) were male. Of all hospitalised patients included in the study, 426 (31.1%) died and 943 (68.9%) were discharged. Baseline patients' characteristics can beseen in Table 1.

There was a statistically significant difference in age, absolute neutrophils, lymphocytes and platelets counts, NLR and PLR between two groups studied. The correlation of C-reactive protein (CPR) with NLR and PLR was verified with Spearman's correlation coefficient. Both were direct correlations with coefficient 0.39 (moderate correlation) and 0.27 (weak correlation), respectively ($p < 0.001$), Figure 1.

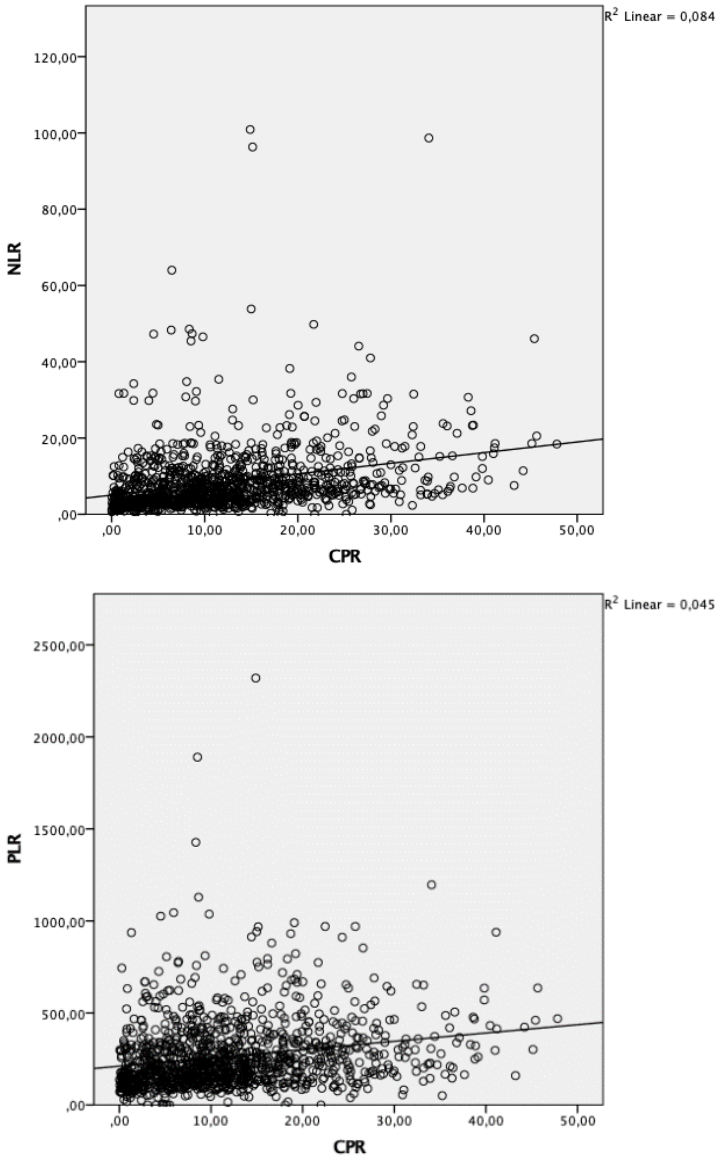

Figure 1 - Correlation of C-reactive protein (CPR) with neutrophil to lymphocyte (NLR) and platelet to lymphocyte (PLR) ratios.

The risk of the clinical outcome was significantly higher in patients with NLR of 3.3 than in those with an NLR< 3.3, p<0.001. (Table 1).

Table 1 - Baseline characteristics – Data are n (%) or median (IQR). Comparison between the two groups were performed using chi-square for categorical variables or Mann-Whitney for continuous variables. Missing data: obesity (n = 572), hypertension (n = 81), diabetes (n = 100), chest tomography involvement (n = 176), CPR (n = 572).

	Whole samples	Group		p
		Death (n = 426)	Hospital discharge (n = 943)	
Age (years) (n = 1369)	68 (59-77)	73 (64-81)	66 (56-75)	<0.001
Male gender (n = 1369)	701 (51.2%)	233 (33.2%)	468 (66.8%)	0.90
Obesity (n = 797)	299 (37.5%)	89 (29.7%)	210 (70.3%)	0.628
Hypertension (n = 1288)	832 (64.5%)	298 (35.8%)	534 (64.2%)	<0.001
Diabetes (n = 1269)	535 (42.1%)	186 (34.7%)	349 (65.3%)	0.007
Chest tomography involvement ≥ 50% (n = 1193)	588 (49.2%)	227 (38.6%)	361 (61.4%)	<0.001
Hemoglobin (g/dL) (n = 1369)	13 (11.6-14.2)	12.8 (11-14.2)	13 (11.8-14.2)	0.025
Neutrophils count (x109/L) (n = 1369)	5.8 (3.9-8.6)	6.5 (4.5-9.5)	5.5 (3.8-8.0)	<0.001
Lymphocyte count (x109/L) (n = 1369)	.97 (.65-1.4)	.80 (.57-1.2)	1.0 (.70-1.4)	<0.001
Platelet count (x109/L) (n = 1369)	213 (164-276.75)	196 (153-269)	219 (171-278)	<0.001
CPR (n = 797)	10.3 (5.2 – 17.3)	13.3 (7.9 – 21.4)	9.1 (4.2 – 15)	<0.001
Neutrophil / lymphocyte ratio (NLR)	6 (3.48-10)	7.67 (4.6-14.5)	5.41 (3.15-9.23)	<0.001
Platelet / lymphocyte ratio (PLR)	213.1 (139.6-327.5)	240 (150-388.2)	210.9 (139.1-309.4)	<0.001
NLR ≥ 3.3	1048 (76.6%)	362 (34,5%)	686 (65.5%)	<0.001
PLR ≥ 180	818 (59.8%)	270 (33%)	548 (67%)	0.074

A binary logistic regression was used to examine factors associated with mortality. A hierarchical method was performed with the variables NLR, previous diagnosis of hypertension, previous diagnosis of diabetes, chest tomography involvement and age. Multicollinearity was assessed by examining the variance inflation factor (values > 2 were excluded). All the variables were predictors of mortality, except previous diagnosis of diabetes (Table 2). The results are presented as odds ratios and 95% confidence intervals.

Table 2 - Binary logistic regression model for predictors of mortality.

Variable	OR	95% CI	p
NLR	1.04	1.03-1.06	<0.001
Hypertension	1.41	1.04-1.91	0.024
Chest tomography involvement ≥ 50%	2.46	1.86-3.24	<0.001
Age	1.04	1.03-1.06	<0.001

DISCUSSION

We evaluated a large number of patients diagnosed with Covid-19 and the hematological parameters showed differences between groups of outcomes of death and hospital discharge. These evidences reinforce the previously described findings [4-12]. Twenty to 40% of COVID-19 patients have leukopenia, and 3–24% have leukocytosis. Lymphopenia (lymphocyte count ≤ 1,100 cells/μL) was seen in 30–75% of COVID-19 patients. A recent meta-analysis found a strong association between lymphopenia and severe COVID-19 [11]. On the other hand, neutrophilia has been reported in severe COVID-19 patients and it may be due to viral-induced inflammation or due to secondary bacterial infections, seen in approximately 10% of COVID-19 patients. Despite significant heterogeneity among the studies, analysis has emphasised that the association between NLR levels on admission and poor outcomes for COVID-19 was independent of predictors, such as age, hypertension, diabetes mellitus, and cardiovascular diseases [9-10]. An elevated neutrophil to lymphocyte ratio was identified as a marker for in-hospital mortality and severe COVID-19 disease [7]. At present, even though neutrophilia was not observed at the first examination of the admission of these patients, there was a statistical significance in the value of initial neutrophils between both groups, reinforcing those higher values of neutrophils are associated with a worse clinical outcome.

Decreased lymphocyte counts were associated with more severe outcomes with p<0.001. The lymphopenia appears to be associated with an invasion of lymphocytes by the virus, as ACE2 receptors are found on lymphocytes. The virus may directly attack lymphocytes causing apoptosis, invade bone marrow cells, or cause destruction of the spleen or lymph nodes [13].

The NLR calculated at hospital admission had high value in predicting the subsequent poor clinical outcomes. We validated a cut off value of 3.3 for NLR, calculate on the first blood count at hospital admission 4-5. The NLR value was higher in group 1 - death than in group 2 - hospital discharge, p <0.001 and, consequently, the presence of cutoff> 3.3 was higher in group 1 (84.9%) than in group 2 (7 .7%), p<0.001.

In this study, a moderate correlation was found between CRP and NLR values, reinforcing the findings already described in the literature [6].

Thrombocytopenia is described in about 5 to 41.7% of patients with COVID-19, with an incidence that varies according to the severity of the disease. It is usually light with

counts usually between 100 to 150 X109/L [7]. Severe thrombocytopenia is rarely reported in patients with COVID-19, for example in association with an immune thrombocytopenic purpura-like state [14]. In the present study, thrombocytopenia was not found in the initial assessmet of the patients, however higher platelet levels were associated with a better outcome in these patients (Hospital discharge group), with p <0.001.

The platelet to lymphocyte ratio is confirmed to be a good candidate for predicting the outcome of patients with different inflammatory diseases, such as neoplasias, acute pancreatitis and cardiovascular disease [7]. The analysis of laboratory values and demographic findings of three hundred and four patients diagnosed with COVID-19 infection showed that the NLR and PLR ratios of those with severe clinical symptoms were significantly higher (p < 0.001), concluding that these ratios can be used as significant biomarkers in predicting the prognosis of patients [15]. The group-2 had higher platelet values when compared to group-1, with the worst evolution (p<0.001). However, the present study failed to demonstrate a correlation between a cut of value of 180 for PLR and better clinical outcome, with no statistical difference between the two groups studied (p=0.074).

CONCLUSION

In conclusion, this study with a large number of evaluated patients reinforces that the identification of effective biomarkers of progressive disease, such as NLR, can be useful for diagnosis, prevention of complications and effective therapy in COVID-19.

LIMITATIONS OF THE STUDY

As this is a retrospective study analysing medical records, which date back to the height of the SARs-Cov 2 pandemic, important variability between reports was found. Thus, the present study is subject to information bias that had to be removed in the analysis, mainly correlated to the periodicity and time of signs and symptoms of the disease and clinical worsening. Furthermore, the date of the first test collection was not the same for all individuals analysed, as the demand for care was heterogeneous, with some seeking care at the onset of symptoms and others later. Therefore, for greater clarity of these findings, further work must be carried out, standardising the date of blood count collection in relation to the onset of symptoms, as well as greater standardisation of signs of clinical worsening.

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