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ORIGINAL ARTICLE

Evaluation of whole blood profile as a tool in COVID-19 diagnosis and screening. A cross-sectional study

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Abstract

Backgroung: the COVID-19 epidemic began in December 2019, and the shortage of diagnostic resources has affected the reported data on the number of cases, resulting in variations in reported cases between countries. This situation underscores the necessity for a deeper understanding of SARS-CoV-2 pathophysiology, including blood profiles and potential predictors.

Methods: hematological variables were studied in 200 patients diagnosed with COVID-19, before the vaccination period started. We analyzed hemogram parameters: erythrocytes, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) mean corpuscular hemoglobin concentration (MCHC), hematocrit, red cell distribution width (RDW), platelets, mean platelet volume (MPV), leukocytes, neutrophils, lymphocytes, neutrophils/lymphocytes ratio and Platelet-to-Lymphocyte Ratio (PLR), up to 9 days after positive result for COVID-19.

Results: the positive COVID-19 group presented a higher mean age, as well as a higher frequency of male individuals. Erythrocyte, hemoglobin, hematocrit and MCH values were significantly lower, while RDW and PLR showed higher values in the positive group. Leukocytes, neutrophil and the neutrophil/lymphocyte ratio presented higher values in the positive COVID-19 group.

Conclusion: data showed that the hemogram, a low-cost, minimally invasive exam, supports diagnosis and screening of COVID-19, allowing better evaluation of the disease course and assisting medical decisions facing lack of resources in a pandemic situation.

Keywords: Hematological profile, Hematimetric index, COVID-19, pandemic, screening.

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Authors summary

Why was this study done?
This study was conducted to assess the importance of a complete blood count (CBC) examination in identifying the hematological profile of patients affected by COVID-19.

What did the researchers do and find?
The data indicated that the hemogram, an affordable and minimally invasive examination, can serve as a valuable tool for screening, diagnosing, and even predicting the prognosis of COVID-19.

What do these findings mean?
A hematological signature was identified in these patients, providing valuable information for treatment decision-making, and contributing to improved post-disease outcome monitoring.

Highlights
The COVID-19 pandemic has amplified the significance of accurate and comprehensive diagnosis, particularly in infectious diseases such as those caused by the SARS-CoV-2 virus.
Additionally, the feasibility of easily manageable and cost-effective diagnostic measures can facilitate monitoring of potential changes in COVID-19 infection.
The hematological profile, as a result of a complete blood count (CBC) examination, is among the tests commonly requested in primary care that can provide extremely important information to elucidate patients' prognosis.
The proper evaluation of the CBC promotes advancements in effectively combating pandemics, making informed decisions in public health, and improving care for patients affected by the disease. In addition to being a low-cost test, it is routinely requested in all medical consultations.

INTRODUCTION

The acute respiratory tract infection epidemic, known as COVID-19, started in December 2019 and its most frequent clinical symptoms are respiratory, such as cough, fever and difficulty breathing. Nevertheless, the clinical aspects of the new corona virus infection (SARS-CoV-2) are wild heterogeneous, ranging from a cold to serious pneumonia[1]. The elderly and individuals with comorbidities (hypertension, chronic obstructive pulmonary disease, diabetes and cardiovascular disease) rapidly evolved to acute respiratory syndrome, septic shock, severe metabolic acidosis and coagulation disfunction, leading to worsening of the condition and death[2,3].

Because of the rapid and global spread, the World Health Organization (WHO) declared in March 11th 2020 the new coronavirus disease (2019-nCoV), a pandemic[4]. However, the divulged data on the number of cases may not represent the actual reality; there are doubts about the COVID-19 mortality rate, because these rates vary between countries. One reason for this variation is the lack of resources such as tests to confirm the infection[5]. Furthermore, there is an alert for asymptomatic cases and patients with mild symptoms, who may not be tested and consequently will not be identified and included in the statistic of COVID-19 confirmed cases. This situation brings to light the necessity of further knowledge on SARS-CoV-2 physiopathology, such as blood profile and possible predictors[6,7].

Among the studied predictors are the vital hematological parameters which include leukocyte, neutrophil, lymphocyte and platelet counts and the proportion between the neutrophil and lymphocyte ratio. Some hematological parameters, like the ones mentioned above, can help in predicting and monitoring the progression of several diseases[8,9].

Just as occurred in severe acute respiratory syndrome (SARS) in 2003 in China, and in Middle East respiratory syndrome (MERS) in 2012, there are alterations in circulating blood cells, besides abnormalities in function and in lymphocyte counts. Leucopenia, neutrophilia and thrombocytopenia were blood alterations frequently detected in positive patients[2,10-12].

The Lymphopenia may be related to an immune response deficiency toward the virus. Neutrophilia can indicate increased cytokines in a hyper-inflammatory state with of abnormalities in cytoplasmatic and nuclear morphology, Ranging from hypo-segmented nucleus to apoptosis[11]. This increases neutrophil/lymphocyte ratio (NLR) which is associated with higher inflammatory cytokines (IL-2, IL-6 and IL-10) and higher IgG values, leading to serious cases and worse prognosis[13]. Regarding platelet count which is drastically decreased in severe patients and especially in non-survivors, a condition thought to result from platelet activation, aggregation, and adhesion as well as increased consumption of these plasmatic elements[14].

Brandon Michael Henry and collaborators observed that severe cases presented a slight increase in leukocyte count while fatal patients showed a significantly higher increase of this parameter. Decreases in hemoglobin and eosinophil counts are also reported[15]. Another study observed that the increase in the Red Cell Distribution Width (RDW) is associated with higher mortality in hospitalized patients with COVID-19, no matter the age. Nevertheless, higher RDW levels at the moment of hospital admission predicted higher mortality rate[16]. Moreover, in severe cases, hemophagocytic syndrome was also observed to be caused by macrophage activity[17]. Lee *et al*. (2021) conducted a meta-analysis, demonstrating the potential usefulness of RDW measurement in predicting COVID-19 mortality and disease severity, highlighting its significance as a prognostic biomarker[18]. Similarly, they retrospectively studied the clinical outcomes of hospitalized COVID-19 patients based on their RDW values, revealing a strong association between elevated RDW and unfavorable outcomes in this patient population[19].

It is known that hematological manifestations are common in COVID-19 disease, but there are still few data available about the prevalence and clinical

significance of anemia and other alterations in this disease. In most cases, anemia was mild and was caused by an inflammatory process, associated or not with iron and vitamins deficiency. Therefore, this condition can have a negative impact on patients' quality of life, so monitoring the Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCM) and Mean Corpuscular Hemoglobin Concentration (MCHC) is of great importance. Even so, data is still inconsistent, and more observations need to be made[20].

Despite of the differing studies over hematological abnormalities, we believe there is still space to explore these parameters given their importance in the current pandemic scenario and the necessity of further knowledge of its repercussion. Furthermore, it is important to consider and study other parameters such as RDW or platelet to lymphocyte ratio (PLR), which is already used for acute pancreatitis, breast cancer and acute myocardial infarction prognostic and as a severity predictor for cirrhosis associated with chronic hepatitis and fibrosis[21].

Considering that a hemogram is a low cost, minimally invasive exam, which is already used as a prognostic tool for acute pancreatitis, breast cancer and acute myocardial infarction. The objective of the present study was mapping the major hematological alterations observed in hospitalized COVID-19 patients, regardless of the severity of the disease, and to identify the most prevalent hematimetric changes in these patients. The results of this study will be fundamental to strengthen the diagnosis in SUS and assist in the monitoring of COVID-19 inpatients, besides aiding medical decisions. This evaluation can also cooperate with possible drug or supplementation studies to improve immunological response and patient recovery.

METHODS

Delineation

This is a cross-sectional study which evaluates hematological variables in 200 patients diagnosed with COVID-19, in comparison with 200 negative patients, between July 2020 and January 2021, before the vaccination period started. This study was conducted during the first wave of COVID-19 in the city of São Bernardo do Campo and Santo André. All participants received care in field hospitals; therefore, patient inclusion followed a nonprobability or nonrandom sampling, considering the pandemic state. As a result, there was no distinction based on the severity level or mortality of the included patients[22]. Reports were collected through the Matrix FMABC platform, in which it is possible to obtain data on all exams performed in Laboratório de Análises Clínicas of Centro Universitário FMABC, taken from samples acquired from health centers in the municipalities of São Bernardo do Campo and Santo André.

Eligibility Criteria

We analyzed hemogram parameters up to 9 days after a positive result for COVID-19, as the disease typically spans a 14-day period, and symptoms typically manifest around the 5th day of infection, prompting individuals to seek healthcare services for testing[23]. Therefore, within the first 9 days after a positive test, patients are still in the active phase of the disease. This study did not differentiate severity or mortality data. Given the convenience sample nature, we did not perform a matching test between female and male participants. Age and sex were not distinguished among the participating groups, as they were selected based on demand in the health services of Santo André and São Bernardo do Campo.

COVID-19 Positive Group: Patients suspected of coronavirus infection confirmed by a positive RT-qPCR test, with no age or sex distinctions. COVID-19 Negative Group: Patients suspected of coronavirus infection ruled out by a negative RT-qPCR test, with no age or sex distinctions. Exclusion criteria: patients with previous coronavirus infection, patients who did not fit the inclusion criteria, patients with hemograms performed over 9 days from the COVID-19 detection RT-qPCR test.

Hematological evaluation

Erythrogram, leukogram and platelet counts were performed by flow cytometry in a ABX Pentra DF 120™ device, following good practices in clinical analysis. The analyzed parameters were specific: erythrocytes (Reference value (RV): for men 4.5 - 5.5 million/mm^3 and for women 4-5 million/mm^3), hemoglobin (RV: for men 13-17 g/dL and for women 12-15 g/dL dL), VCM (RV: 80-100 µm^3), MCH (RV: 27-32 pg), MCHC (RV: 31.5-36 g/dL), hematocrit (RV: 36 -46%), RDW (RV: 12-15%), platelets (RV: 150-400 103 mm^3), MPV (RV: 6-10 µm^3), leukocytes (RV: 4, 5-11 103 mm^3), neutrophils (RV: 2-8 103 mm^3), lymphocytes (RV: 1-5 103 mm^3) and the ratio between neutrophils and lymphocytes, which is a marker for reflecting the inflammatory status of the patient and serves as a prognostic factor[24,25].

Ethical aspects

This study is in accordance with Process N° 466 de 12/12/2012, from Conselho Nacional de Saúde – CNS, which regulates research with humans. The project was approved by Comitê de Ética em Pesquisa - CEP from FMABC, nº 4.427.013, as laid down in the 1964 Declaration of Helsinki.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). The unpaired Student t-test was performed on normal distribution values and the Mann Whitney test was performed on nonparametric distribution values[26]. These analyses were performed in GraphPad Prism program (GraphPad, version 7.0, USA). The significant level established was 5% (descriptive value of $p < 0.05$).

RESULTS

We observed a predominance of females in the negative COVID-19 group, corresponding to 61,5% of total patients. Male predominance occurred in the positive COVID-19 group with 63% of total infected patients. The mean age in the negative COVID-19 group was 44 ± 18 years, while the positive COVID-19 group presented a significantly higher mean, of 52 ± 19 years.

In positive COVID-19 patients lower values of erythrocytes was observed in comparison to the negative

COVID-19 group, both between men (positive: 4.5 ± 0.8 vs. negative: 4.9 ± 0.4, CI 95%, $p < 0.05$) and between women (positive: 4.1 ± 0.8 vs. negative: 4.4 ± 0.3, CI 95% $p < 0.05$) (figures 1A and B). Hemoglobin also presented lower values in COVID-19 patients both between men (positive: 13.4 ± 2.4 vs. negative: 14.8 ± 1, CI 95%, $p < 0.05$) and between women (positive: 11.7 ± 2.4 vs. negative: 13.3 ± 0.9 CI 95%, $p < 0.05$), as shown in figures 1C and 1D.

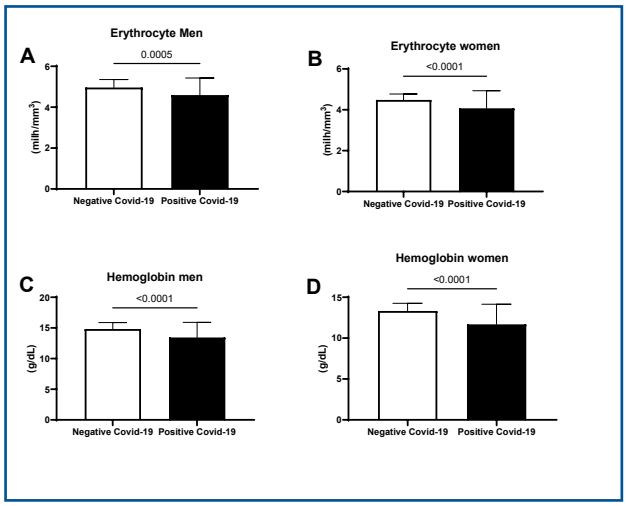

Figure 1: Representative graph of erythrocytes values in men (1A) and women (1B). Hemoglobin in men (1C) and in women (1D) in COVID-19 positive patients versus COVID-19 negative patients.
Student t-test * $p < 0.05$ vs. negative COVID-19, CI=95%. Data expressed as mean ± SD.

Positive patients with COVID-19 also presented significantly lower values of hematocrit both between men (positive: 39.4 ± 6.7 vs. negative: 43.2 ± 2.9, CI 95%, $p < 0.05$) and between women (positive: 34. ± 7.1 vs. negative: 38.8 ± 3.5, CI 95%, $p < 0.05$) (figure 2A and B).

RDW values were also higher in positive patients (positive: 13.89 ± 2.036 vs. negative: 13.133 ± 0.919, CI 95%, $p < 0.05$), as displayed in figure 2C. With regard to platelets, there was no statistical difference between groups (figure 2D).

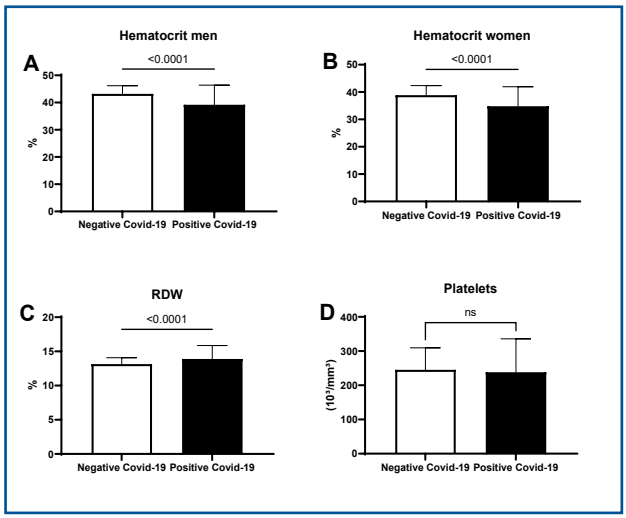

Figure 2: Representative graph of hematocrit (2A and B), RDW (2C) and platelet (2D) values in COVID-19 positive patients versus COVID-19 negative patients.
Student t-test * $p < 0.05$ vs. negative COVID-19, CI=95%. Data expressed as mean ± SD.

Lower MCH values were observed in positive COVID-19 patients in comparison to negative patients (positive: 29.15 ± 2.74 vs. negative: 29.8 ± 1.73, CI 95%, $p < 0.05$) (figure 3B). There were no statistical differences in VCM or MCHC between groups (figure 3A and 3C).

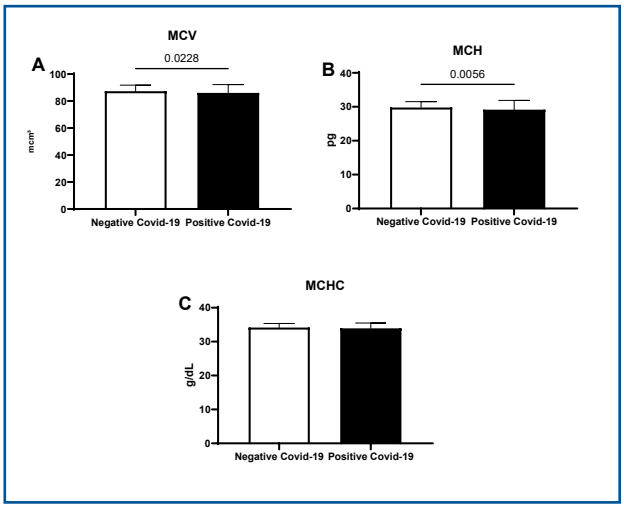

Figure 3: Representative graphs of MCV (3A), MCH (3B) and MCHC (3C) values in COVID-19 positive patients versus COVID-19 negative patients.
Student t-test * $p < 0.05$ vs. negative COVID-19, CI=95%. Data expressed as mean ± SD.

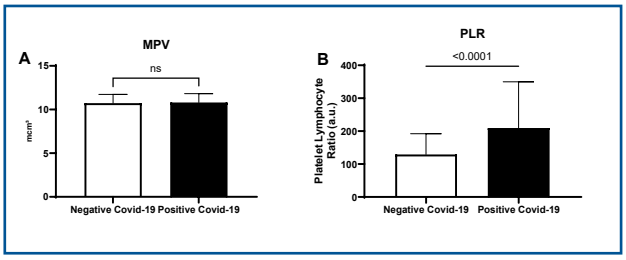

Figure 4: Representative graphs of MPV values (A) and PLR values (B) in COVID-19 positive patients versus COVID-19 negative patients.
Student t-test * $p < 0.05$ vs. negative COVID-19, CI=95%. Data expressed as mean ± SD.

When compared to other hematological parameters, patients with COVID-19 presented higher leukocyte values (positive: 9.563 ± 5.633 vs. negative: 8.042 ± 1.877, CI 95%, $p < 0.05$) and neutrophil (positive: 7.028 ± 4.852 vs. negative: 4.892 ± 1.637, CI 95%, $p < 0.05$) as reported in figures 5A and 5B. Regarding lymphocyte values, no statistical difference between studied groups was found (figure 5C). The neutrophil/lymphocyte ratio was also higher in positive patients (positive: 6.738 ± 6.558 vs. negative: 2.758 ± 1.906, CI 95%, $p < 0.05$), as depicted in figure 5D.

DISCUSSION

The present study evaluated hematological variables in patients diagnosed with COVID-19 and in uninfected patients, and all samples originated from the public health system. The period of collections was before the start of vaccinations. In these circumstances we observed significant lower values for erythrocytes in infected patients, which is associated with abnormalities in erythrocyte structure due to codification of some non-

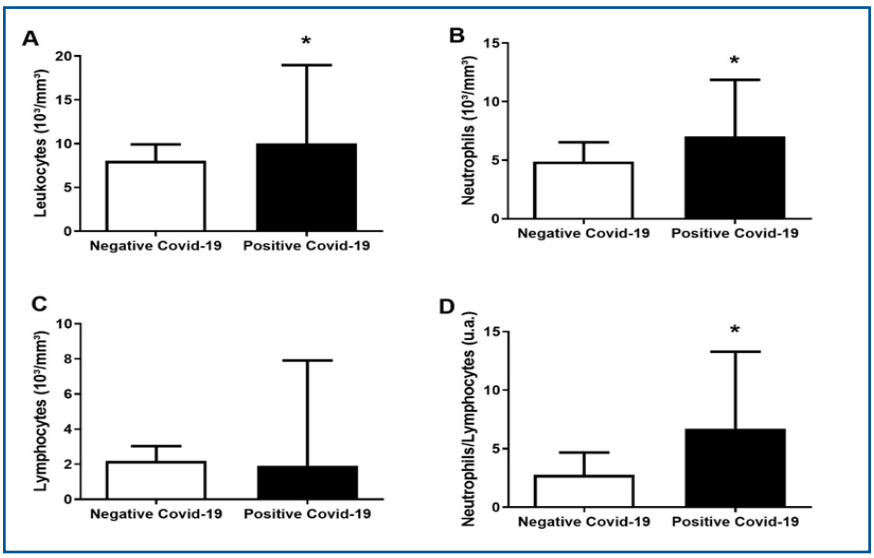

Figure 5: Representative graphs of leukocytes (5A), neutrophils (5B), lymphocytes (5C) and neutrophil/ lymphocyte ratio (5D) values in COVID-19 positive patients versus COVID-19 negative patients.
Student t-test * $p < 0.05$ vs. negative COVID-19, CI=95%. Data expressed as mean ± SD.

structural proteins by viral RNA.

Moreover, in advanced stages of the disease an elevation was already observed in macrophage activity stimulated by cytokines, being equally harmful to erythrocytes. Furthermore, we observed a decrease in hemoglobin in which there are also structural impacts with removal of the iron atom caused by viral protein synthesis, impairing oxygen transport[2]. As expected, this alteration was reflected in hematocrit values which were significantly lower in positive COVID-19 patients as well. Progressive decrease in erythrocytes and hemoglobin may indicate worsening of the clinical course of the disease. Besides, significantly lower values of MCH were found in infected patients, indicating hypochromic anemia, due to low concentration of hemoglobin per red blood cell[10].

Our study also observed higher values of RDW in positive COVID-19 patients, in concordance with as described by Foy *et al*. in a prospective study, where elevated RDW at hospital admission and increasing RDW during hospitalization were associated with higher mortality risk in these patients[16]. Hypoxia is believed to be one of the possible causes of the rise in this parameter since in order to compensate lack of oxygen there is an increase in erythropoietin and consequently in RDW, through mechanisms involving erythrocyte regulation of maturation and survival processes. This data reinforces that the increase in RDW may have an important role in the early detection of hypoxemia in positive COVID-19 patients; hence it is suggested that this parameter be closely monitored in these patients[27].

In this study we observed elevated neutrophil values in positive patients. This increase is known to be a consequence of the cytokine storm caused by a hyperinflammatory state in SARS-CoV-2 infection, in addition to also being an indicator of concomitant bacterial infection. Likewise, we have obtained significantly lower values of leukocytes in positive COVID-19 patients which can be indicative of associated bacterial infection as well[11].

Lymphopenia is a common finding in patients infected by SARS-CoV-2, probably because of an alteration in immune response due to viral infection[28]. Even though, in this study there was no statistical difference in lymphocyte counts between the groups. It was verified that lymphocyte values may vary according to disease course time[11]. Therefore, lymphocyte variability may possibly be associated with time of collection, since in this study the results were not related to disease progression.

We also evaluated neutrophil / lymphocyte ratio (NLR) which was significantly higher in positive COVID-19 patients, corroborating with the fact that NLR is already identified as a predictive and prognostic indicator of COVID-19 severity[13].

Regarding platelet counts, there was no difference between the studied groups. Some studies describe lower platelet counts associated with higher mortality; however, many severe positive COVID-19 patients did not present this alteration. Despite this, as platelets have active participation in immune response, changes in their numbers and activity should be carefully analyzed[29].

The MPV is an important parameter that has been studied in various clinical contexts, including infectious processes. Assessing MPV in evaluations of infectious processes can provide valuable insights into the body's response to infection and inflammation. Nordin *et al.*, (2004)[24] conducted a multicentre study of reference intervals for haemoglobin, basic blood cell counts, and erythrocyte indices in the adult population of the Nordic countries, providing non-parametric reference intervals for B-Haemoglobin, B-Erythrocytes, B-EVF, B-MCV, Erc-MCH, and other hematological parameters. These reference intervals serve as valuable benchmarks for assessing hematological parameters, including MPV, in the context of infectious processes. Korniluk *et al.*, (2019)[30] discussed the new perspectives for an old marker in the course and prognosis of inflammation, emphasizing the role of MPV as a biomarker in various inflammatory conditions, including infectious diseases, diabetes mellitus, myocardial infarction, and cancer. In summary, the

assessment of MPV in the context of infectious processes provides valuable information about platelet activity and the body's response to infection, contributing to a better understanding of the hematological changes associated with infectious diseases.

The PLR has been extensively studied as an inflammatory marker and a potential indicator of disease activity in various medical conditions. Kim *et al.* (2015)[31] assessed the utility of PLR in patients with psoriasis vulgaris and psoriatic arthritis, highlighting its potential as an inflammatory marker in these conditions. Similarly, Qin *et al.* (2016)[32] demonstrated the usefulness of PLR as a marker for assessing inflammatory response and disease activity in patients with systemic lupus erythematosus (SLE).

The correlation between PLR and COVID-19 infection has been examined. Sarkar *et al.* (2021)[33] conducted a systematic review and meta-analysis to assess the link between PLR at admission and outcomes, including mortality and severity among COVID-19 patients, offering valuable insights into the prognostic significance of PLR in COVID-19. Erdogan *et al.* (2021)[34] investigated the prognostic role of PLR in COVID-19 patients, contributing insights into the utility of PLR as a prognostic indicator in this patient population. Aksu *et al.* (2021)[35] explored the predictive role of PLR in assessing both lung involvement and severity in patients with COVID-19, providing valuable insights into the potential clinical applicability of PLR in evaluating COVID-19 severity.

The evaluation of PLR obtained by this study showed significantly higher values in positive COVID-19 patients compared to negative ones. Thus, PLR could be considered an important biomarker for prognostic estimations in SARS-CoV-2 infection, considering this parameter is associated with systemic inflammatory response. Nevertheless, this data needs further investigation concerning comorbidities. Additionally, new studies are needed to assess the applicability of this marker in the development of comorbidities post SARS-CoV-2 infection[21].

Epidemiological studies have highlighted a link between SARS-CoV-2 infection and an increased risk of conditions such as cardiovascular diseases, diabetes, neurological disorders, and psychiatric disorders. Understanding these complex interactions is crucial for mitigating the long-term adverse effects of the disease.

Moreover, it is crucial to consider the role of individual patient characteristics, such as age, sex, pre-existing conditions, and immune response, in susceptibility to and manifestation of COVID-19-associated comorbidities. A thorough understanding of these alterations is essential to inform decision-making regarding appropriate disease control and management measures[35-38].

In this study comparing the blood profile of positive versus negative COVID-19 patients, infection severity was not considered, also, the samples were not collected at a specific time of the disease course, these being the biases of this research. Among the study limitations, the lack of discrimination regarding the severity of the disease stands out. It is acknowledged that randomization based on the level of infection involvement could provide more accurate information on the evaluated indices and biomarkers. At first, the objective was mapping the major hematological alterations, before discriminating the severity of the disease, willing to describe a panel of this disease.

CONCLUSION

This study evidenced alterations in hematological parameters of positive COVID-19 patients. These data showed that the hemogram, a low-cost and minimally invasive exam, can support for screening, diagnosis and even prognosis of COVID-19, allowing better evaluation of the disease course and assisting medical decisions facing lack of resources in the pandemic setting.

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Author Statements

All authors participated in the study development and agreed about the publication.

Conflict of interest

The authors declare no conflicts of interest.

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Resumo

Introdução: a epidemia de COVID-19 teve início em dezembro de 2019 e a falta de recursos diagnósticos impactou os dados divulgados sobre o número de casos, resultando em variações nos casos relatados entre os países. Essa situação destaca a necessidade de um conhecimento mais profundo sobre a fisiopatologia do SARS-CoV-2, como o perfil sanguíneo e possíveis preditores.

Método: variáveis hematológicas foram estudadas em 200 pacientes diagnosticados com COVID-19, antes do início do período de vacinação. Analisamos os parâmetros do hemograma: eritrócitos, hemoglobina, hemoglobina corpuscular média (HCM), concentração média de hemoglobina corpuscular (CMHC), hematócrito, amplitude de distribuição dos glóbulos vermelhos (RDW), plaquetas, volume plaquetário médio (VPM), leucócitos, neutrófilos, linfócitos, relação neutrófilos/linfócitos e razão plaquetas/linfócitos (RPL), até 9 dias após o resultado positivo para COVID-19.

Resultados: o grupo positivo para COVID-19 apresentou uma média de idade mais alta, assim como uma frequência maior de indivíduos do sexo masculino. Os valores de eritrócitos, hemoglobina, hematócrito e HCM foram significativamente menores, enquanto o RDW e o PRP apresentaram valores mais altos no grupo positivo. Os leucócitos, neutrófilos e a relação neutrófilos/linfócitos apresentaram valores mais altos no grupo positivo para COVID-19.

Conclusão: os dados mostraram que o hemograma, um exame de baixo custo e minimamente invasivo, apoia o diagnóstico e a triagem da COVID-19, permitindo uma melhor avaliação da evolução da doença e auxiliando nas decisões médicas diante da falta de recursos em uma situação de pandemia.

Palavras-chave: perfil hematológico, indice hematimétrico, COVID-19, pandemia, triagem.