SAS Journal of Medicine
Abbreviated Key Title: SAS J Med
ISSN 2454-5112
Journal homepage: https://saspublishers.com

OPEN ACCESS

Medicine

Biomarkers Predicting the Severity of COVID-19

Zakaria Chahbi[1*], M. Raiteb[1], L. Abainou[2], M. Mouharir[1], I. Belatik[1], M. Badaoui[1], S. Kaddouri[1], H. Qacif[1], M. Zyani[1]

[1]Internal Medicine Department, Avicenna Hospital, Marrakesh, Morocco
[2]Endocrinology Department, Avicenna Hospital, Morocco

DOI: 10.36347/sasjm.2023.v09i06.016 | **Received:** 03.03.2023 | **Accepted:** 12.04.2023 | **Published:** 10.06.2023

***Corresponding author:** Zakaria Chahbi
Internal Medicine Department, Avicenna Hospital, Marrakesh, Morocco

Abstract **Original Research Article**

COVID -19- has been the cause of a tremendous sanitary crisis throughout the world. The different studies that have been conducted to identify the main effectors of COVID-19, emphasized on the key role of the Cytokine Storm and the hypercoagulable state in poor outcome. This is why the analysis of the biomarkers is crucial to categorize the patients as soon as they are admitted so we can improve their medical care. A retrospective study was performed at the military hospital of Marrakesh, Morocco, which included 435 patients with COVID-19, from 1st July to 31st December. Patients were divided in severe cases (250 cases) and non-severe cases (180 cases). We explored age, sex, comorbidities presence as well as the following biomarkers: Hemoglobin, Platelet count, Leucocytes, Neutrophils, Eosinophiles, Lymphocytes, Neutrophil to Lymphocyte ratio NLR, Platelet to Lymphocyte ratio PLR and CRP. The biomarkers the showed the largest area under the curve AUC were NLR (0,909), neutrophils (0,850), CRP (0,810) PLR (0,769) and leucocytes (AUC>0.773). NLR was identified as the most performant biomarker for predicting severe outcome in COVID-19. The cut-off value displaying the greatest prediction was determined to be 3,9.

Keywords: Covid 19-Biomarkers, Neutrophil Lymphocyte ratio, Platelet Lymphocyte ratio, Morocco.

INTRODUCTION

Since December 2019, the emergence of a new strain of coronavirus called coronavirus SARS-CoV-2 has been identified as the causative agent of a new respiratory disease: COVID-19 [1].

Symptoms can range from a simple classic flu-like illness of cough, fever or arthralgia, to moderate or severe pneumonia. High-flow oxygen therapy or respiratory assistance is then necessary to improve the vital prognosis. In addition, there is a disturbed biological assessment, reflecting an excessive inflammatory response, qualified as a real cytokine storm [2]. This storm is the consequence of an unbalanced innate and adaptive immune response, responsible for irreversible tissue damage leading to multiorgan failure.

In addition to the clinical signs, biological markers or biomarkers reflect the excessive inflammatory response, but also the immune status of the host. They are used to guide therapy but also to predict the severity of COVID-19 pneumonia [3]. Among these markers, we find: CRP, hemoglobin level, platelets, leukocytes and its subgroups (neutrophils, eosinophils and lymphocytes) and finally the NLR (Neutrophil Lymphocyte ratio) and PLR (Platelet Lymphocyte ratio) ratios. .

Our study was motivated by the increase of declared positive COVID-19 cases in our country, during the period following mid-July 2020. Because this time, and contrary to the beginning of the crisis, more severe and fatal forms have been observed [4]. The objective of our study was to analyze all of these biological parameters and to determine the most sensitive and specific to predict the severity of the disease.

MATERIAL AND METHODS

This is a retrospective analytical study carried out within the hospital's isolation services, between July 01, 2020 and December 31, 2020. It included 435 cases infected with the SARS-CoV2 virus, diagnosed by RT - PCR on nasopharyngeal samples. We divided our sample into two distinct populations: one presenting a severe form (255 cases) and another is presenting a non-severe form (180 cases).

The severity criteria were defined as follows: the presence of respiratory symptoms on admission

consisting of dyspnea, polypnea or desaturation requiring the use of high-flow oxygen or even a transfer to intensive care for ventilation, as well as the all patients whose symptoms worsened during their hospitalization. Non-severity criteria include symptoms such as fever, dry cough, asthenia, anosmia or ageusia without hemodynamic or respiratory repercussions. The biological analyzes taken into account were those carried out on admission for each patient. We excluded any patient who received corticosteroid therapy before admission to the service that could influence their blood test (false hyperleukocytosis).

Data entry and statistical analysis were carried out jointly using IBM SPSS statistics (27.0) and Excel. The value of the optimal thresholds of the biomarkers was calculated using the ROC curve (Receiver Operating Characteristic curve). The relative risk and the confidence interval were defined at 95%. The significant threshold is retained for a $p<0.05$.

RESULTS

In this study we included 435 patients. The average age was 59 years old. The sex ratio was 3.14 (330 men versus 105 women). In this series, 207 patients had one or more comorbidities, including 129 severe cases. The most frequent of these comorbidities is diabetes, with a percentage of 42%, followed by hypertension with 17%.

The number of severe cases identified in our population is 255 cases, or almost 59% of the population, against 180 non-severe cases, 41% of the population. The total number of deaths is 42 deaths, or almost 10% of the population studied.

Distribution of biomarker values in severe and non-severe cases is summarized in Tables I and II.

Table I: Summary table of the medians of the study variables

Severity	Non-severe cases		Severe cases	
	Median + IQ	N	Median + IQ	N
Age (years)	59[49-65]	180	59[45-67]	255
Comorbidities		78		129
Hemoglobin (g/dl)	14,3[12,7-15,3]	180	13,4[12,4-14,7]	252
Platelets (/mm³)	198500[157000-246000]	180	227000[155750-294500]	254
Leukocytes (/mm³)	5850[4690-7140]	179	8645[6230-11315]	254
Neutrophil (/mm³)	3415[2555-4490]	180	6950[4790-9690]	255
Eosinophil (/mm³)	20[0-70]	180	10[0-20]	255
Lymphocytes (/mm³)	1630[1290-2105]	180	990[730-1340]	255
CRP (mg/l)	17,7[3,1-57,4]	177	106,8[51,9-200]	245
NLR	2,1[1,5-2,5]	180	6,5[4,5-10,2]	255
PLR	120,4[86,3-162,8]	180	213,2[150,6-345]	254

Table II: Summary table of biological anomalies

	Non-severe cases		Severe cases	
	%	Total	%	Total
Anemia (<12 g/dl)	25	180	11,37	255
Thrombocytopenia (<150000/mm³)	29,44	180	14,11	255
Leukopenia (<4000/mm³)	11,36	176	2,4	250
Hyperleukocytosis (>10000/mm³)	3,4	176	34,8	250
Neutropenia (<2500/mm³)	2,22	180	0,78	255
Neutrophilia (>7500/mm³)	3,33	180	40,4	255
Eosinopenia (<40/mm³)	65,1	178	83,46	254
Lymphopenia (<1500/mm³)	39,44	180	80	255
Increased CRP (>5mg/l)	68	176	95	244

To study the specificity and sensitivity of the biomarkers, we used the ROC curve (Figure 1), which allowed us to specify the area under the curve of each element, called "AUC". The AUC of the curve of the biomarker studied must be greater than or equal to 0.5 in order for it to perform well.

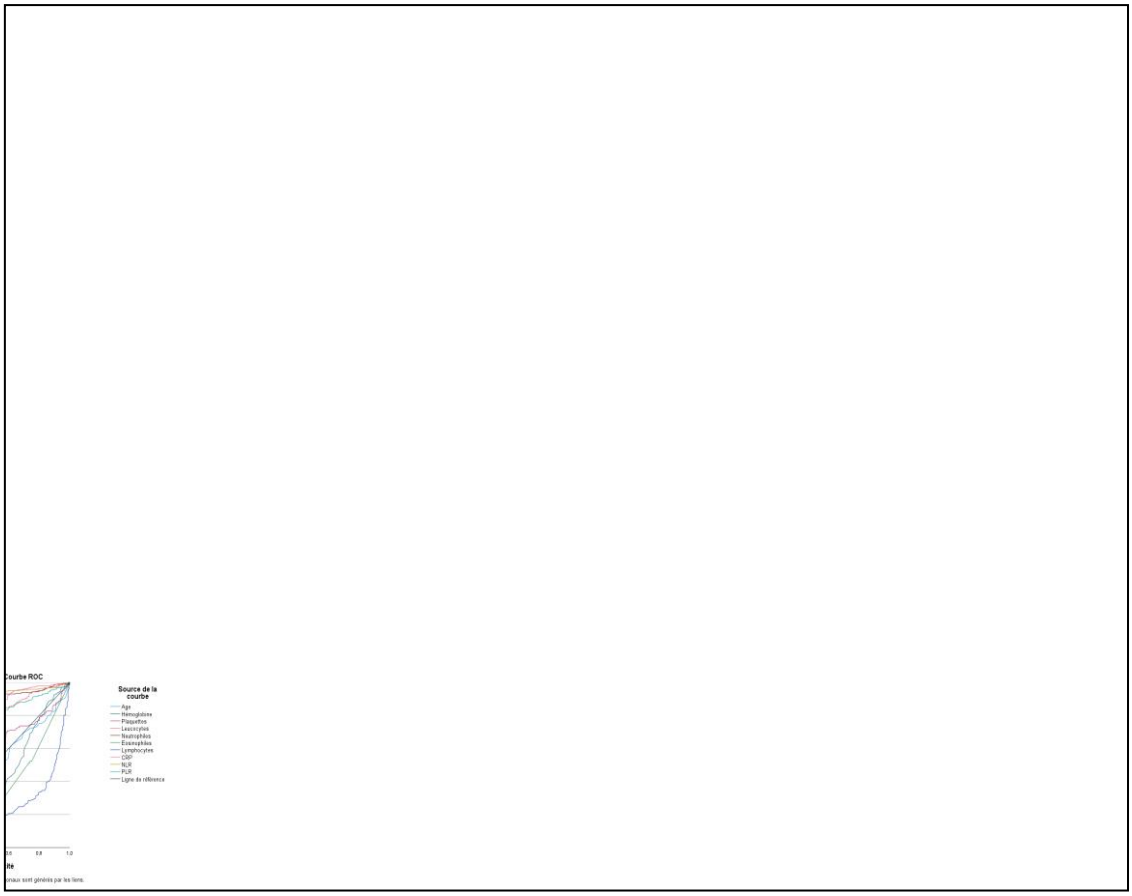

Figure 1

The optimal thresholds predictive of severity found through ROC curve analysis are: 3.9 for the NLR, 185.3 for PLR, 7515/mm³ for leukocytes, 4710/mm³ for neutrophils and 72 mg/l for CRP.

The best performing parameter in predicting the severity of Covid19 according to our study is the NLR ratio. The ranking of other biomarkers predictive of Covid19 severity according to their performance is summarized in Table (III).

Table III: Ranking and cutoff value of biomarkers predictive of COVID-19 severity

Biomarker	Threshold value	Classification by performance
NLR	3,9	1
Neutrophiles	4710/mm3	2
CRP	72 mg/l	3
PLR	185,3	4
Leukocytes	7515/mm3	5

DISCUSSION

Although the mechanism of inflammation during COVID-19 is not fully elucidated, most data in the literature agree on the observation of an inappropriate immune response, characterized by the release of inflammatory cytokines, such as TNF (Tumor Necrosis Factor), NK (Natural Killers) cells, interleukins, monocytes/macrophages, as well as the main agents of adaptive immunity; T and B cells [5]. The severity of COVID-19 appears to be modulated not only by the viral infection itself but also by an aberrant host immune and inflammatory response. Recent data suspect that this immune dysregulation is involved in the host immunosuppression phase [6] which occurs after the pro-inflammatory or "cytokine storm" phase. The latter is characterized by peripheral lymphopenia and an increased risk of bacterial superinfection [7].

The NLR (Neutrophil Lymphocyte Ratio) is the number of peripheral neutrophils divided by the number of peripheral lymphocytes. The PLR (Platelet Lymphocyte ratio) is the number of peripheral platelets divided by the number of peripheral lymphocytes. Both are biomarkers of systemic inflammation in the host [8]. Frequently used in clinical practice, these biomarkers are quick and inexpensive to calculate and have a variety of uses in cardiovascular, pulmonary cancer and other pathologies [9].

The median NLR ratio in the severe population is 6.5 [4.5-10.2] compared with 2.1 [1.5-2.9] in the non-severe population. Our results are similar to those of the Spanish study by Ruiz *et al.*, which found a ratio of 2.07 in the non- severe cases against a ratio of 6.58 in the severe cases [10] Lopez Escobar *et al.*, [11] found higher values of 8.7 [4.3- 14.3] for non-survivors versus 3.8 [2.5-6.7] for survivors. In our study, the NLR ratio is considered the best performing biomarker of severity. The predictive threshold value is 3.9.

This result is comparable to the series of Yang *et al.*, [12], whose cut-off value is 3.3 for the occurrence of severity.

The systematic review by Li *et al.*, which grouped 13 studies dealing with the predictive role of the NLR ratio in the occurrence of severity, highlighted the performance of the NLR ratio (AUC= 0.78) and determined a threshold of 4.5 as the cut-off value for severity. A ratio slightly increased compared to that of our study [13]. Ponti *et al.*, found an NLR ratio of 2.973 as the optimal threshold beyond which the evolution is unfavorable [14]. The NLR ratio is increased if the neutrophil count is increased or if the lymphocyte count is decreased. These two abnormalities are the most common in severe COVID-19 infection as described in our study. The increased NLR ratio in COVID-19 patients could be explained by the existence of non-functional endothelial tissue during viral infection, which would promote cell damage or even cell apoptosis. This tissue would be correlated with the existence of comorbidities such as diabetes, hypertension or cardiovascular diseases [10]. The median PLR ratio in the severe population is 213.2 [150.6-345] versus 120.4 [86.3- 162.8] in the non-severe population. Ratios comparable to those of the study by Ruiz *et al.*, [10]: PLR equal to 200 in the population classified as severe versus 193.3 in that classified as non-severe. Yang *et al.*, [12] found significantly higher results: 436.5 in severe cases versus 176.7 in non-severe cases. In our study, the predictive threshold value for severity is 185.3 (AUC> 0.773). A result similar to that of Yang *et al.*, [12]: a threshold of 180 (AUC>0.784). In sum, the NLR ratio, PLR ratio, WBC and neutrophil counts, and CRP are the best performing biomarkers for predicting the severity of COVID-19 in our study using ROC curve analysis. In other words, a patient with one of the above-mentioned biomarkers above the threshold predicted in our results is more likely to have a severe form of COVID-19.

CONCLUSION

Our study, the first of its kind in our country, analyzed the most common biological biomarkers, namely hemoglobin, platelets, white blood cells and their subgroups, and NLR, PLR, and CRP ratios. We were able to determine the most predictive biomarkers of COVID-19 severity, namely NLR ratio, PLR ratio, white blood cell count, and CRP, and their predictive threshold. Finally, the NLR ratio was retained as the best performing biomarker with a minimum threshold value of 3.9, which is predictive of severity.

Conflict of Interest: None.

REFERENCES

1. Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., ... & Tan, W. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *New England journal of medicine*, 382(8), 727-33.
2. Hu, B., Huang, S., & Yin, L. (2020). The cytokine storm and COVID-19. *J Med Virol*, [Internet]. [cité 11 juin 2021]; Disponible sur https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7361342/
3. TSOUMBOU-BAKANA, G., TRAORE, B., HASSOUNE, S., & Samira, NANI (2020). BIOLOGICAL FACTORS PREDICTIVE OF SERIOUS FORMS OF COVID 19. *Moroccan Review of Public Health*, *7* (11), 13-7.
4. Covid-19 : au Maroc, les hôpitaux craignent d'être submergés par la deuxième vague. Le Monde.fr [Internet]. 18 nov 2020 [cité 11 juin 2021]; Disponible sur: https://www.lemonde.fr/afrique/article/2020/11/18/covid-19-au-maroc-les-hopitaux-craignent-d-etre-submerges-par-la-deuxieme-vague_6060253_3212.html
5. Fu, Y., Cheng, Y., & Wu, Y. (2020). Understanding SARS-CoV-2-Mediated Inflammatory Responses: From Mechanisms to Potential Therapeutic Tools. *Virol Sin*, 35(3), 266-71.
6. Djavadi-Ohaniance, L., & Hatefi, H. (1975). Oxydation of NADPH by submitochondrial particles from beef heart in complete absence of transhydrogenase activity from NADPH to NAD. *J Biol Chem*, 250(24), 9397-403.
7. Moroi, K., & Sato, T. (1975). Comparison between procaine and isocarboxazid metabolism in vitro by a liver microsomal amidase-esterase. *Biochem Pharmacol*, 24(16), 1517-21.
8. Fest, J., Ruiter, T. R., Groot Koerkamp, B., Rizopoulos, D., Ikram, M. A., van Eijck, C. H., & Stricker, B. H. (2019). The neutrophil-to-lymphocyte ratio is associated with mortality in the general population: The Rotterdam Study. *European journal of epidemiology*, *34(5)*, 463-470.
9. Afari, M. E., & Bhat, T. (2016). Neutrophil to lymphocyte ratio (NLR) and cardiovascular diseases: an update. *Expert Rev Cardiovasc Ther*, 14(5), 573-7.
10. Ruiz. eci.13404.pdf.
11. López-Escobar, A., Madurga, R., Castellano, J. M., Ruiz de Aguiar, S., Velázquez, S., Bucar, M., ... & Ventura, P. S. (2021). Hemogram as marker of in-hospital mortality in COVID-19. *Journal of Investigative Medicine*, *69*(5), 962-969.

12. Yang, A. P., Liu, J. P., Tao, W. Q., & Li, H. M. (2020). The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. *International immunopharmacology*, *84*, 106504.
13. Li, X., Liu, C., Mao, Z., Xiao, M., Wang, L., Qi, S., & Zhou, F. (2020). Predictive values of neutrophil-to-lymphocyte ratio on disease severity and mortality in COVID-19 patients: a systematic review and meta-analysis. *Critical Care*, *24*(1), 1-10.
14. Ponti, G., Maccaferri, M., Ruini, C., Tomasi, A., & Ozben, T. (2020). Biomarkers associated with COVID-19 disease progression. *Critical reviews in clinical laboratory sciences*, *57*(6), 389-399.