Original Article

Role of Hematological and Inflammatory Markers in Early Diagnosis and Severity of COVID-19 Disease

Seema Awasthi, Ankita Mittal, Vivek Singh, Ashutosh Kumar, Faiyaz Ahmad, Nilisha Sharma

Department of Pathology, Teerthanker Mahaveer Medical College and Research Center, Moradabad, Uttar Pradesh, India

Abstract

Background: COVID-19 virus, causing severe acute respiratory illness (SARS-CoV-2), was declared as a pandemic by the WHO in March 2020, after its first outbreak in China at the end of 2019. The major purpose is to establish the role of a hematological and inflammatory markers in early diagnosis of COVID-19 illness and its relationship with the disease severity. **Materials and Methods:** The study was performed in a tertiary care center from April to September 2020. The study included 150 hospitalized COVID-19 Reverse transcription-polymerase chain reaction positive patients. According to ICMR standards, research patients were grouped into mild, moderate, and severe categories depending on clinical evaluation. Different laboratory parameters complete blood counts, prothrombin time (PT), activated partial thromboplastin time (APTT), d-dimer, serum ferritin, C-reactive protein (CRP), and mean results are compared among the patient in three disease severity groups. **Results:** In the studied population, there were 106 (70.7%) males and 44 (29.3%) females. The average age of the research participants was 48.40 ± 11.50 (21–75 years), with majority of patients being old (>60 years). Hematological markers such as total leukocyte count, Neutrophil-to-lymphocyte ratio (NLR), and platelet lymphocyte ratio (PLR) and the levels of PT, APTT, and D-dimer, as well as ferritin and CRP, all were considerably high with different groups of disease severity ($P = 0.001$). **Conclusion:** The study concluded that patients of severe disease category have significantly higher levels of leukocytosis, neutrophilia, elevated NLR, PLR, PT, APTT, D-dimer, serum ferritin, and CRP. Hematological and coagulation symptoms are associated with COVID-19 illness, and these indicators might be employed as a prognosticator for prediction of early disease severity.

Keywords: Coagulation profile, COVID-19, hematological parameter

Introduction

COVID-19 is a coronavirus-2-related viral illness that produces acute respiratory illness with severe symptoms (SARS-CoV-2). After first being come across in China (Wuhan city, Hubei province) at the end of 2019, the WHO mentioned it a pandemic in March 2020.[1,2]

Inflammation is linked to increasing infectious diseases, and evidence suggests that here too it is crucial in the pathogenesis of viral pneumonia, including COVID-19. The activated macrophages produce cytokines and chemokines as a result of cellular damage caused by SARS-COV2 viral replication. As a result, immunological responses are triggered, resulting in cytokine storms and aggravation. Because of inflammatory process, blood tests play a significant role in early illness diagnosis. Leukocyte count and features such as neutrophil or lymphocyte predominance, inflammation C-reactive protein (CRP), damage to other organs (kidney and liver), and illness severity are all included in these tests of investigation. Furthermore, biomarkers provide information about the nature of pneumonia, allowing the clinician to establish whether the condition is caused by bacteria or by other causes.[3]

Complete blood counts (CBC) are a simple and least expensive test to perform. Total leucocyte count (TLC), number of neutrophil, lymphocyte, platelet, mean platelet volume (MPV), and specific ratios of these data are all included in the CBC. Neutrophils are the most distinguishable white blood cell

Submitted: 25-Mar-2022 **Revised:** 06-May-2022
Accepted: 14-Jun-2022 **Published:** 27-Jun-2022

Access this article online

Quick Response Code:

Website: www.actamedicainternational.com

DOI: 10.4103/amit.amit_39_22

Address for correspondence: Dr. Ankita Mittal, Associate Professor, Department of Pathology, Teerthanker Mahaveer Medical College & Research Centre, Moradabad - 244 001, Uttar Pradesh, India.
E-mail: dhanyamittal09@gmail.com

How to cite this article: Awasthi S, Mittal A, Singh V, Kumar A, Ahmad F, Sharma N. Role of hematological and inflammatory markers in early diagnosis and severity of COVID-19 disease. Acta Med Int 2022;9:73-7.

type which form an important component of inflammatory processes and are regulated by mast cells, epithelial cells, and macrophages. Lymphocytes play an important function in both inflammation and infection. Furthermore, thrombocytes have a role in the control of many inflammatory processes. While any of these characteristics can be utilized as an inflammatory marker on its own, their ratios to one another can also be employed as early indicators of inflammation.[4] Stress causes circulating leukocytes to increase neutrophils and decrease lymphocytes; the ratio of these two characteristics is employed as an inflammatory measure.[5]

The adaptive immune response is determined by the strength of the inflammatory reaction, resulting in an imbalanced immunological response. A greater risk of severe COVID-19 infection has been found associated with increased serum ferritin, erythrocyte sedimentation rate (ESR), CRP, and interleukin-6 (IL-6) levels.[6-8]

Circulating biomarkers with information on inflammation and immune function might be helpful in predicting the prognosis of COVID-19 patients. Platelet lymphocyte ratio (PLR) and Neutrophil-to-lymphocyte ratio (NLR) are markers of systemic inflammation.[9,10]

They have also been studied extensively in a variety of disorders, including cancer (including hematological cancers), respiratory, gastrointestinal, cardiovascular, intracerebral hemorrhage, and systemic diseases. Higher readings have been linked to more serious diseases with the worst prognosis.[11]

These hematological and inflammatory markers, such as CBC, D-dimer, CRP, serum ferritin, and coagulation tests, might have a significant impact on the prediction of early illness severity and can help to reduce disease morbidity and mortality by providing a better guide for prompt patient management. The purpose of this study is to observe any correlation between these hematological and inflammatory markers and the disease severity.

Materials and Methods

Study design

This retrospective cross-sectional research performed at a Tertiary care facility at TMMC and RC Moradabad in the Department of Pathology.

Study setting

The data utilized in this study has been anonymized to protect the identities of individuals involved. The study was conducted from April 2020 to September 2020. Research was approved by Institutional Ethical Committee (TMU/IEC/21/145).

Sample size

The study comprised a total of 150 reverse transcription-polymerase chain reaction (RT-PCR) positive confirmed cases. Research participants were categorized as mild, moderate, and severe according to ICMR standards for COVID-19 patients.[12]

Method of data collection

Comparison of different laboratory parameters was performed among these 3 categories. A CBC was conducted on Sysmex XN-10, complete coagulation profile on fully automated immunoassay Vidas while CRP was measured on semi-automated analyzer 300 and serum ferritin was measured on the fully automated immunoassay. We studied parameters such as white blood cells (WBC), NLR, PLR, Platelet count, MPV, red blood cells (RBC), Hemoglobin (Hb), prothrombin time (PT), activated partial thromboplastin time (APTT), D-dimer, serum ferritin, and CRP.

Statistics

Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS) for Windows (version 25.0). Collected data was analyzed by SPSS (version 25.0) (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). A P value <0.001 was considered statistically significant.

Results

In our study, out of a total of 150 cases, 55 cases (36.66%) were classified as severe, 45 cases (30%) as moderate and 50 cases (33.33%) as mild disease according to ICMR guidelines.

In the studied population, there were 106 (70.7%) males and 44 (29.3%) females. As indicated in Table 1, our study found a male preponderance of 106 (70.7%) cases and a female accounted for 44 (29.3%) cases within the study group.

As indicated in Table 1, the patient age ranged 21–75-year-old, with average age of the study group being 48.40 ± 11.50. Majority of research participants are in older age group (>60 years), indicating that illness severity rises with age.

All laboratory parameters in the three groups of disease severity are shown in Table 2 indicating differences in their mean values. Hematological parameters such as WBC, TLC, NLR, PLR, PT, APTT, serum ferritin, CRP, and D-dimer were significantly increased in patients of severe category of disease as compared to mild and moderate group of disease and showed statistical significance with disease severity (P < 0.001) whereas other parameters such as RBC, hemoglobin, platelet count (PC), and MPV showed no significant difference among different categories of disease.

Table 1: Gender and age distribution of patients

Age group (years)	Number of cases (150)	Male	Female
<30	15	10	5
31-60	80	46	34
>60	55	50	5
Mean±SD	48.40±11.50	106 (70.7%)	44 (29.3%)

SD: Standard deviation

Table 2: Laboratory parameters according to disease severity

	Mean±SD			P
	Mild cases (50 cases)	Moderate cases (44 cases)	Severe cases (55 cases)	
Test				
RBC	4.33±0.85	4.35±1.05	5.28±5.34	0.26
HB	12.29±2.07	12.22±2.60	12.03±2.57	0.32
TLC	9477.20±4562.66	14573.68±9343.42	16563.18±7366.35	0.001
NLR	28.82±2.62	28.74±3.27	30.64±2.16	0.001
PLR	146.54±95.24	199.73±152.67	237.3±28.2	0.001
PLT	2.03±0.94	1.99±1.27	2.53±1.01	0.289
MPV	12.11±1.47	12.49±1.62	11.87±1.58	0.329
Coagulation profile				
PT	24.91±79.66	52.84±44.46	64.72±60.93	0.001
APTT	39.75±36.64	54.66±35.07	55.04±39.38	0.001
D-Dimer	2032.97±2388.0	2773.03±4795.33	3010.93±3135.87	0.001
Ferritin	150.18±151.88	548.15±360.47	557.20±414.0	0.001
CRP	21.20±39.28	38.08±89.61	55.72±177.43	0.001

SD: Standard deviation, RBC: Red blood cells, HB: Hemoglobin, TLC: Total leukocyte count, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, PLT: Platelet, MPV: Mean platelet volume, PT: Prothrombin time, APTT: Activated partial thromboplastin time, CRP: C-reactive protein

Discussion

COVID-19 causes a systemic infection that affects the entire body and has a major influence on the immunological and hematopoietic system. ESR, CRP, Serum ferritin, alpha 1 antitrypsin, hepcidin, haptoglobin, cytokines, and IL-6 are various examples of indicators of systemic inflammation. The function of these hematological and biochemical indicators in diagnosis and prognosis of COVID-19 has been explored. We test CRP, S. ferritin, D-dimer, and hematological markers because they are least expensive, easy to obtain, and provide data quickly. We compared our findings to those of other researchers around the world.

The findings of our investigation revealed that the hematological and hemostatic manifestations of COVID-19 patients as well as their relationship to the severity of disease, were related with the disease severity.

Among the various predictors for COVID-19, increasing age was a significant factor and associated with poor outcomes. Most of the cases in our study belong to the elderly age group with mean age of 48.40 ± 11.50 (21–75) with male preponderance (70.7% males) which correlates with the study conducted by Singh *et al*.,[4] Wang *et al*.[13] and Jin *et al*.[14] The primary goal of our research was to determine the predictive importance of laboratory test performed on COVID-19 patients which comprised of hematological, coagulation profile, and key indicators of inflammation. In terms of hematological parameters, our study revealed higher levels of TLC with statistical significance ($P < 0.001$) with the severity of disease, similar findings were observed by Qin *et al*.,[15] Huang *et al*.[16] and Zhang *et al*.[17]

In our study, we observed increase in NLR ratio with statistical significance ($P < 0.001$) which was due to increase in absolute neutrophil count (ANC) and a decrease in absolute lymphocyte count, similar findings were reported by Qin *et al*.,[15] Huang *et al*.[16] and Zhang *et al*.[17] Since our study also shows neutrophilia due to increase in ANC which corresponds to Hu *et al*.[18] findings. The most noticeable finding especially among severe cases was lymphocytopenia which was due to affinity of coronavirus for lymphocytes angiotensin-converting enzyme receptors causes cytopathic impact and lymphocyte death due to which neutrophilia was seen as an inflammatory response, resulting in lymphocytopenia. However, the PC did not differ substantially across the groups which was similar with the findings of Archana *et al*.,[10] Fan *et al*.[19] and Ferrari *et al*.[5] but in our study PLR was on higher side with statistical significance ($P < 0.001$). Gong *et al*.[20] discovered the same findings which they attribute to lymphocytopenia. RBC, Hb, and MPV had no correlation with the disease severity in our study.

The screening test for aberrant clotting is the coagulation profile which includes PT, APTT, and D-dimer levels. In our research, we noticed that coagulation profile varied substantially according to the illness severity. We found that PT and D-dimer were increased from mild to severe disease and showed statistical significance ($P < 0.001$) between the three groups which was comparable with the findings of Huang *et al*.[16] and Tang *et al*.[21] The presence of a D-dimer indicates active fibrinolysis and, as a result, coagulation. It evaluates the severity of the host's reaction. D-dimer was evaluated in all the cases in our study but elevated levels were detected in 94.6% of cases, which was comparable with other studies Han *et al*.,[22] Siordia.[23] and Tang *et al*.[21] We found a substantial rise in D-Dimer levels from mild to severe cases which were statistically significant as well ($P < 0.001$) which was comparable with the studies by Gao *et al*.[24] and Archana *et al*.[10] who also found a difference that is statistically

significant in mild and severe group ($P < 0.007$ and < 0.0021), respectively. According to a study by Bhutta *et al*.[25] septic shock and risk of sepsis in patients increases with rise in D-dimer level. The D-dimer found to be an excellent predictor of disease progression.

CRP is produced by the liver against the inflammatory or infectious conditions are an acute phase reactant. CRP levels in plasma are very stable, unlike other acute-phase proteins, which exhibit significant fluctuations in plasma level (based on the rate of synthesis, consumption, and catabolism). Serum concentrations rise rapidly during acute inflammation makes it a more reliable indication of sepsis.[26] CRP plays an important role in the pro-inflammatory cycle by triggering the production of inflammatory cytokines in the body.

Initial increase CRP levels were linked to outcomes in clinical practice shown in are search by Smilowitz *et al*.[27] Patients with higher CRP levels were at increased risk of venous thromboembolism, acute renal damage, severe illness, and fatality. Our study revealed raised CRP levels in 73.2% of cases which was comparable with other studies such as Mardani *et al*.,[6] Li *et al*.,[28] Mo *et al*.,[29] and Chen *et al*.[30] (77.1%, 44%, 100%, and 93.1% cases, respectively). Guan *et al*.[31] from China also showed similar results with elevated CRP level in 60.7% of cases.

We also found out increase in the levels of CRP from mild, moderate to severe cases and showed statistically significant ($P < 0.001$) which is comparable with findings of Gao *et al*.[24] and Mo *et al*.[29]

Serum ferritin is produced in the body during various inflammatory diseases (infections, hematologic, neoplastic, and rheumatologic). The serum iron status in the body can impact ferritin levels, which can suggest a hyperimmune condition. It is a sign for hemophagocytic lymphohistiocytosis, a well-known viral infection consequence.[32,33]

In our research, we found a considerable variation with increase in ferritin level from mild to severe cases which was statistically significant ($P < 0.001$) which is line with the findings of Sun *et al*.[32] who found same results ($P < 0.000$). Archana *et al*.[10] ($P < 0.02$) and Mehta *et al*.[3] found similar results, observed a considerable difference in ferritin levels between survivors and nonsurvivors groups.

Serum ferritin and CRP levels were considerably higher in severe category cases compared to mild and moderate categories in our research. Terpos *et al*.[34] revealed elevated ferritin and lactate dehydrogenase levels were the risk factors for acute respiratory distress syndrome (ARDS), intensive care unit (ICU) support, and mortality in a retrospective cohort analysis from Wuhan, China. Raised CRP has also been linked to the development of ARDS, higher Troponin-T levels, cardiac damage, and fatality in COVID-19 patients.

D-dimer, ferritin, and CRP are useful in risk classification, prognosis prediction and therapeutic therapy. In COVID-19 patients routine monitoring appears to be recommended.

Conclusion

Thus we conclude that the patient with COVID-19 has higher hemocytometric alteration (such as Raised TLC, NLR, PLR, and ANC) as well as lymphocytopenia. Significant elevations of coagulation profile (PT, APTT, and D-dimer) are observed in severe category cases. Inflammatory indicators such as CRP, ferritin are also considerably elevated in patients of COVID-19. Based on our findings, we proposed that routine laboratory parameters along with CRP, Ferritin, and D-dimer have the ability to detect, predict prognosis, consequences, and monitoring therapy response.

In symptomatic instances where molecular assays are unavailable or there is a risk of false-negative RT-PCR cases, high suspicion of COVID-19 infection is recommended based on these laboratory data. These data can be utilized as a screening tool to distinguish individuals who are at early risk of serious illness and might require ICU and proper monitoring to decrease the burden of tertiary care hospital in terms of personnel resources, oxygen bed, and other logistical issues. This could also aid in early prevention, detection, proper treatment and thus lowering morbidity and mortality related with COVID-19 infection.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al*. A novel corona virus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727-33.
2. World Health Organization. WHO Corona Virus Disease (COVID19) Dashboard. Geneva: World Health Organization; 2020. Available from: https://covid19.who.int [Last accessed on 2021 Nov 15].
3. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, *et al.* COVID-19: Consider cytokine storm syndromes and immunosuppression. Lancet 2020;395:1033-4.
4. Singh P, Kumar A, Singh S, Kelkar A, Doshi P, Nimbargi RC, *et al.* Utility of routine haematological parameters and infectious biomarkers to assess the disease severity in COVID19 positive patients, analysis and early trend from India.10 July 2020, Preprint(Version 1) available at Research Square[http:// doi:10.21203/rs.3.rs-40378/v1].
5. Ferrari D, Motta A, Strollo M, Banfi G, Locatelli M. Routine blood tests as a potential diagnostic tool for COVID-19. Clin Chem Lab Med 2020;58:1095-9.
6. Mardani R, Ahmadi Vasmehjani A, Zali F, Gholami A, Mousavi Nasab SD, Kaghazian H, *et al.* Laboratory parameters in detection of COVID-19 patients with positive RT-PCR; a diagnostic accuracy study. Arch Acad Emerg Med 2020;8:e43.
7. Najim R, Kadhi S. Biochemical and hematological parameters as apredictor for COVID-19 infection in 65 patients diagnosed by realtime–PCR in Kirkuk city. Syst Rev Pharm 2020;11:797-9.
8. Agrawal A, Tyagi P, Mahavar S, Banerjee S, Sharma R, Bhandhari S. Study of hematological and biochemical parameters in a cohort of Indian COVID-19 patients admitted in a tertiary care centre. Int J Adv Med 2020;7:1840-5.

9. Henry BM, de Oliveira MH, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): A meta-analysis. Clin Chem Lab Med 2020;58:1021-8.
10. Archana B, Shyamsunder S, Das R. Validity of Markers and Indexes of Systemic Inflammation in Predicting Mortality in COVID-19 infection: A Hospital based Cross Sectional Study. J Card Pulm Rehabil 2021;5:146.
11. 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 2020;395:497-506.
12. Clinical Management Protocol: Covid 19. Delhi: Government of India, Ministry of Family Welfare; 2020. Available from: http://www.mohfw.gov.in/pdf/UpdatedClinicalManagementProtocolforCOVID19dated03072020.pdf.[Last accessed on 2021 Nov 15].
13. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al*. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323:1061-9.
14. Jin JM, Bai P, He W, Wu F, Liu XF, Han DM, *et al.* Gender differences in patients with COVID-19: Focus on severity and mortality. Front Public Health 2020;8:152.
15. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, *et al.* Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis 2020;71:762-8.
16. Huang J, Cheng A, Lin S, Jhu Y, Chen G. Individualized prediction nomograms for disease progression in mild COVID-19. J Med Virol 2020;92:2074-80.
17. Zhang G, Hu C, Luo L, Fang F, Chen Y, Li J, *et al*. Clinical features and outcomes of 221 patients with COVID-19 in Wuhan, China. J Clin Virol 2020;127:104364.
18. Hu L, Chen S, Fu Y, Gao Z, Long H, Ren HW, *et al*. Risk factors associated with clinical outcomes in 323 COVID19 patients in Wuhan, China. Clin Infect Dis 2020;71:2089-98.
19. Fan BE, Chong VC, Chan SS, Lim GH, Lim KG, Tan GB, *et al.* Hematologic parameters in patients with COVID-19 infection. Am J Hematol 2020;95:E131-4.
20. Gong J, Ou J, Qiu X, Jie Y, Chen Y, Yuan L, *et al*. A tool to early predict severe 2019-novel coronavirus pneumonia (COVID19): A multicenter study using the risk nomograms in Wuhan and Guangdong, China. Clin Infect Dis 2020;71:833-40.
21. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel corona virus pneumonia. J Thromb Haemost 2020;18:844-7.
22. Han H, Yang L, Liu R, Liu F, Wu KL, Li J, *et al.* Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. Clin Chem Lab Med 2020;58:1116-20.
23. Siordia JA Jr. Epidemiology and clinical features of COVID-19: A review of current literature. J Clin Virol 2020;127:104357.
24. Gao Y, Li T, Han M, Li X, Wu D, Xu Y, *et al.* Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. J Med Virol 2020;92:791-6.
25. Bhutta ZA, Basnyat B, Saha S, Laxminarayan R. Covid-19 risks and response in South Asia. BMJ 2020;368:m1190.
26. Fan SL, Miller NS, Lee J, Remick DG. Diagnosing sepsis – The role of laboratory medicine. Clin Chim Acta 2016;460:203-10.
27. Smilowitz NR, Kunichoff D, Garshick M, Shah B, Pillinger M, Hochman JS, *et al.* C-reactive protein and clinical outcomes in patients with COVID-19. Eur Heart J 2021;42:2270-9.
28. Li LQ, Huang T, Wang YQ, Wang ZP, Liang Y, Huang TB, *et al.* COVID-19 patients' clinical characteristics, discharge rate, and fatality rate of meta-analysis. J Med Virol 2020;92:577-83.
29. Mo P, Xing Y, Xiao Y, Deng L, Zhao Q, Wang H, *et al.* Clinical characteristics of refractory coronavirus disease 2019 in Wuhan, China. Clin Infect Dis 2021;73:e4208-13.
30. Chen L, Liu HG, Liu W. Analysis of clinical features of 29 patients with 2019 novel corona virus pneumonia. Zhonghua Jie He He Hu Xi Za Zhi 2020;43:203-8.
31. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, *et al.* Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708-20.
32. Grangé S, Buchonnet G, Besnier E, Artaud-Macari E, Beduneau G, Carpentier D, *et al.* The use of ferritin to identify critically ill patients with secondary hemophagocytic lymphohistiocytosis. Crit Care Med 2016;44:e1045-53.
33. Sun Y, Dong Y, Wang L, Xie H, Li B, Chang C, *et al*. Characteristics and prognostic factors of disease severity in patients with COVID19: The Beijing experience. J Autoimmun 2020;112:102473.
34. 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;95:834-47.