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Research Article

Association Of Disease Severity And Clinical Outcomes With Circulating Markers Of Netosis And Pyroptosis In SARS-COV-2 Infection.

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Running Title: NETosis and Pyroptosis in COVID-19.

Abstract

Objective: Various cellular pathologies in leukocytes contribute to the pathology of COVID-19 caused by SARS-CoV-2 infection. In this study, we aimed to determine the prognostic significance of netotic and pyroptotic cell deaths in relation to disease severity in COVID-19 pathophysiology.
Materials and Methods: Serum samples were collected from a total of 150 patients with mild, moderate, and severe COVID-19, as defined by the COVID-19 diagnosis and treatment guidelines, along with 89 healthy individuals. Circulating markers of netosis and pyroptosis, including neutrophil elastase (NE), citrullinated histone H3 (CitH-H3), myeloperoxidase (MPO), cell-free DNA (cfDNA), gasdermin D (GSDMD), IL-18, and IL-8, were analyzed using the ELISA method. The effects of netosis and pyroptosis markers on disease severity and clinical parameters were comparatively evaluated, and their associations with inflammation, thrombosis, and fibrinolysis were also investigated.
Results: NE, GSDMD, IL-18, MPO, CITH3, D-dimer, leukocyte and lymphocyte levels, as well as inflammatory markers (CRP, ferritin), showed a significantly elevated in severe COVİD-19 patients compare to the other groups.
Conclusion: Our findings indicate that circulating netosis and pyroptosis markers in SARS-CoV-2 infections correlate with inflammation, dysregulated hemostasis, and fibrinolysis markers, playing a role in disease prognosis. These associations may provide potential therapeutic targets for the treatment of severe COVID-19 cases.

Keywords : SARS-CoV-2, netosis, pyroptosis.

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is one of the most significant global health crises, leading to a wide range of clinical symptoms and affecting multiple organs [1]. In recent years, the rapid emergence of various SARS-CoV-2 variants has sustained viral transmission, resulting in a total of 775 million confirmed cases and over 7 million reported deaths worldwide as of July 22, 2024 (https://covid19.who.int/, accessed April 29, 2023). Since the initiation of mass vaccination in December 2020, overall mortality and morbidity have significantly decreased [2]. However, the emergence of new SARS-CoV-2 variants capable of evading vaccine-induced immune protection remains a clinical and public health concern, as it continues to pose a risk of severe COVID-19 requiring hospitalization, even in vaccinated individuals [3-5]. Research conducted in 2024 highlights that COVID-19 remains a serious health issue, significantly impacting disease progression. Despite ongoing efforts to control viral spread,

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studies indicate that COVID-19 still presents a substantial threat, with some patients experiencing long-term health complications even after recovering from the acute phase. This underscores the fact that COVID-19 is not merely an acute illness but also a disease with long-term health consequences. Most COVID-19 patients exhibit mild clinical symptoms such as fever, chills, and typical respiratory distress [6]. However, in severe cases, a systemic hyperimmune response can emerge, leading to cytokine storms, thrombus formation, and vascular dysfunction, which may result in acute respiratory failure, organ dysfunction, and death [5].

Netotic and pyroptotic cell deaths play a crucial role in viral infections. Netosis is a defense mechanism employed by neutrophils as part of the immune response, particularly during infections. Among the factors contributing to immune dysregulation in COVID-19, neutrophil activation—specifically, the release of neutrophil extracellular traps (NETs)—has garnered significant attention [7,8]. Pyroptosis is a form of cell death triggered by inflammasome activation. During viral infections, pyroptosis can lead to the death of infected cells and the release of pro-inflammatory cytokines such as IL-1β and IL-18. While these cytokines help the immune system combat infections, they can also contribute to excessive inflammation and tissue damage [9].

Netosis is a specialized cellular mechanism by which neutrophils combat pathogens, including bacteria, viruses, and fungi. It is typically triggered by pathogen recognition, as well as by certain cytokines and other stimuli. Upon detecting pathogens such as SARS-CoV-2, neutrophils release NETs—composed of chromatin (DNA and histone proteins) along with various enzymes and antimicrobial proteins—into the extracellular space. The primary function of NETs is to trap and neutralize pathogens, thereby helping to control the infection. NET components can directly kill pathogens or restrict their spread [10,11]. While netosis is a critical defense mechanism, its dysregulation or excessive activation is implicated in a variety of diseases, including autoimmune disorders, inflammatory conditions, infectious diseases, and cancer [12]. Some studies suggest that netosis may play a role in severe COVID-19 pathogenesis [13]. Although NETs are crucial for pathogen neutralization, their uncontrolled release can contribute to inflammation, thrombosis, and tissue damage. Excessive NET formation has been observed in lung biopsies of severe COVID-19 patients, exacerbating pulmonary inflammation and worsening respiratory symptoms and outcomes [7,14]. Additionally, studies have linked excessive NET formation with poor prognosis and increased disease severity in COVID-19 patients [15-18]. Elevated levels of netosis markers—including cell-free DNA (cfDNA), citrullinated histone H3 (CitH-H3), and myeloperoxidase (MPO) as well as the cell death marker lactate dehydrogenase (LDH), have been reported in severe cases. Moreover, serum from COVID-19 patients has been shown to induce netosis in vitro in neutrophils derived from healthy donors [7].

Another form of pathogen-associated cell death, pyroptosis, occurs primarily in myeloid cells and is characterized by cell swelling, plasma membrane rupture, and the release of pro-inflammatory intracellular contents. Pyroptosis is triggered by inflammasome activation in response to pathogens or cellular damage. Its dysregulation is implicated in infectious diseases, autoimmune disorders, and various inflammatory conditions. While excessive or prolonged pyroptosis can lead to tissue damage and inflammation, insufficient pyroptosis may compromise the host's ability to control infections [19]. SARS-CoV-2 has been associated with pyroptosis induction through inflammasome activation in host cells [20]. Studies suggest that SARS-CoV-2 infection can trigger pyroptosis in immune cells, leading to the release of pro-inflammatory cytokines such as IL-1β and IL-18, thereby contributing to the cytokine storm observed in severe COVID-19 cases [19,21].

The dysregulation of netosis and pyroptosis in COVID-19 patients highlights the complex interaction between the virus and the host immune response.

This study aims to investigate the relationship between disease severity and clinical outcomes with netotic and pyroptotic cell death in SARS-CoV-2-infected patients, whose prognosis varies depending on disease severity. By identifying key biomarkers, this research seeks to contribute to understanding the mechanisms underlying COVID-19 pathophysiology and to improving prognostic assessments for affected patients.

MATERIALS AND METHODS

Study Design

This study was conducted using serum samples obtained from 150 COVID-19 patients treated at the COVID-19 Outpatient Clinic, Infectious Diseases Department, Intensive Care Unit, and Emergency Medicine Department of Kanuni Sultan Süleyman Training and Research Hospital during the COVID-19 pandemic. Additionally, 89 healthy volunteers who applied to the Tissue Typing Laboratory of the Department of Medical Biology at Istanbul Faculty of Medicine were included as the control group. The patient group was classified based on disease severity into mild (outpatients, n=50), moderate (hospitalized patients, n=50), and severe (intensive care unit patients, n=50) categories. The classification of disease severity was performed by Kanuni Sultan Süleyman Training and Research Hospital in accordance with the clinical management guidelines of the National Institutes of Health (NIH) and the World Health Organization (WHO).

Inclusion criteria consisted of individuals aged 18 years or older who tested positive for SARS-CoV-2 via real-time polymerase chain reaction (RT-PCR). Exclusion criteria included individuals

with human immunodeficiency virus (HIV), hepatitis B virus (HBV), or hepatitis C virus (HCV) infections, as well as pregnant individuals. The control group consisted of healthy volunteers aged 18 years or older with no known chronic diseases.

Ethical Approval

This study was conducted in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki and its subsequent amendments. Ethical approval was obtained from the Ethics Committee of Biruni University (Approval number and date: 2022/66-10, January 20, 2022). No animal studies were conducted by any of the authors in this research. Informed consent was obtained from all participants.

Sample Preparation

Blood samples were collected in sterile tubes with anticoagulant properties and centrifuged to separate the serum. Serum samples were stored at -80°C until analysis. Before batch analyses, samples were thawed at 37°C for 15 minutes to remove cryoprecipitates.

ELISA Analysis

Serum levels of NE, MPO, cfDNA, CitH-H3, gasdermin-D (GSDM-D), IL-18, and IL-8 were analyzed using enzyme-linked immunosorbent assay (ELISA). ELISA plates were pre-coated by the kit manufacturer (SunLong Biotech Co., LTD), and serum samples were added to the respective microplates. Plates were then washed with a washing solution, secondary antibody conjugates were added, and the washing process was repeated. The reaction was initiated by adding a substrate solution, and results were obtained by measuring the absorbance values of ELISA plates (SunLong Biotech Co., LTD). The absorbance value of each sample reflected the antigen concentration. Statistical analyses were performed to evaluate the potential relationship between COVID-19, netosis, and pyroptosis.

Statistical Analysis

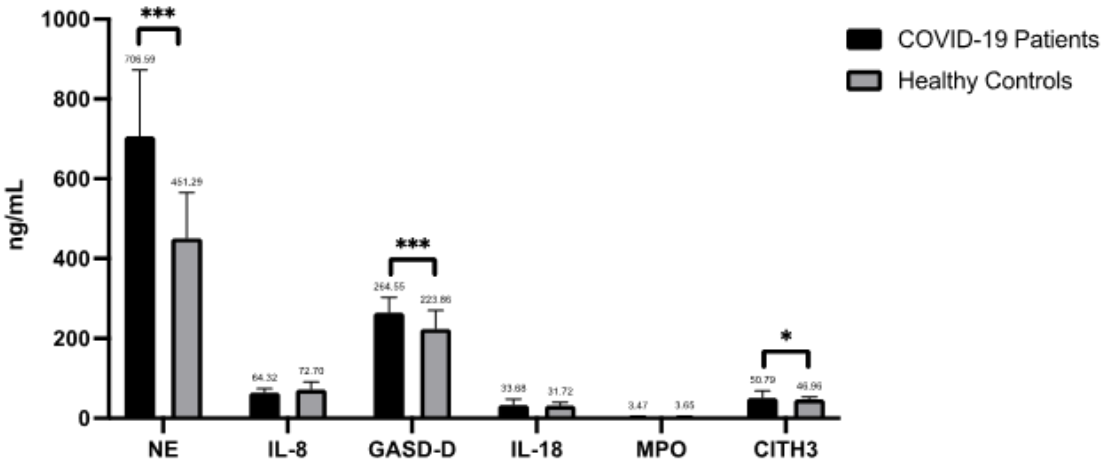
For data with a normal distribution, an independent samples t-test was applied, whereas for non-normally distributed data, the Mann-Whitney U test was used. In tables, normally distributed data were presented as mean ± standard deviation, while non-normally distributed data were presented as median (interquartile range, IQR). Statistical significance was set at $p < 0.05$.

RESULTS

This study assessed the impact of disease severity on inflammatory and cell death markers in individuals with SARS-CoV-2 infection.

The mean age of COVID-19 patients was 53.09±15.12 years, while the mean age of healthy controls was 36.63±12.45 years ($p = 0.0001$). 45,3% of COVID-19 patients were female and 54,7% were male while 52,8 % of healthy controls were female and 47,2% were male. No statistically significant difference was found between the groups in terms of gender. Among patient groups, the most common comorbidities identified were metabolic diseases (29.3%), hypertension (25.3%), and respiratory system diseases (12.7%). The intubation rate was recorded as 21.9%, and the mortality rate was 18.7% (**Table 1**). The levels of netosis and pyroptosis markers (NE, IL-8, GASD-D, IL-18, MPO, CITH3, and cfDNA) were compared between COVID-19 patients and the healthy control group (**Figure 1**). NE, GASD-D, and CITH3 levels were found to be statistically significantly higher in COVID-19 patients compared to the control group ($p<0.05$). In contrast, no significant differences were observed between the two groups regarding IL-8, IL-18, MPO, and cfDNA levels.

Figure 1. Comparison of Serum NET Markers Between COVID-19 Patients (n=150) and Healthy Control Group (n=89). NE (Neutrophil Elastase), GASD-D (Gasdermin-D), and CITH3 (Citrullinated Histone H3) levels were found to be significantly higher in COVID-19 patients ((CITH3 *p<0.05, GASD-D ***p<0.001, NE *p<0.001). No statistically significant differences were observed between the groups for IL-8, IL-18, MPO, and cfDNA levels. Data are presented as median(IQR) values.



Differences in demographic characteristics and comorbidities were also observed among outpatient, hospitalized, and intensive care patients. The mean ages of the patients were 46.02 ± 14.00 in the outpatient group, 53.90 ± 12.95 in the hospitalized group, and 59.36 ± 15.47 in the intensive care group, and the difference in age distribution among the groups was found to be statistically significant (p = 0.000). Regarding gender distribution, the female/male ratios in the outpatient, hospitalized, and intensive care groups were 60/40%, 42/58%, and 34/66%, respectively, and this difference was also statistically significant (p = 0.028) (**Table 1**).

The presence of comorbidities was detected at rates of 44%, 64%, and 62% in the outpatient, hospitalized, and intensive care groups, respectively, and this difference was not found to be statistically significant (p = 0.085). Although metabolic diseases, hypertension, respiratory diseases, cardiovascular diseases, and renal failure were observed at higher rates in intensive care patients, the difference among the groups was not significant. Additionally, neurological diseases, autoimmune diseases, and genetic disorders were detected at lower rates, and the distribution of these diseases did not show statistically significant differences among the groups (**Table 1**).

Table 1. Demographic Characteristics and Comorbidities Among Outpatient, Hospitalized, and Intensive Care Patients.

Characteristics	All COVID-19 Patients (n=150) (%)	Outpatient Patients (n=50) (%)	Hospitalized Patients (n=50) (%)	Intensive Care Patients (n=50) (%)	p-value
Age	53.09±15.12	46,02 ± 14,00	53,90±12,95	59,36±15,476	0.000
Gender (Female/Male)	45,3/54,7	60/40	42 /58	34/66	0.028
Comorbidity	56,7	44	64	62	0.085
Metabolic Disease	29,3	26	34	28	0.658
Hypertension	25,3	18	26	32	0.271
Respiratory Disease	12,7	8	10	20	0.154
Cardiovascular Disease	8,7	6	6	14	0.260
Renal Failure	8	4	6	14	0.149
Neurological Disease	4,7	-	6	8	0.143
Cancer	6,7	8	10	2	0.248
Autoimmune Disease	5,3	8	4	4	0.590
Genetic Disorder	0,7	-	-	2	0.365
Death	21,9	-	-	56	-
Intubation	18,7	-	-	64	-

When netosis and pyroptosis markers, along with other biochemichal parameters were compared among outpatient, hospitalized, and intensive care patients (**Table 2**), NE, IL-18, MPO, CITH3, D-dimer, leukocyte and lymphocyte levels, as well as inflammatory markers (CRP, ferritin), showed a significant association with disease severity. The NE level was found to be significantly lower in intensive care patients (p = 0.002). IL-18 and CITH3 levels were higher in the hospitalized and intensive care groups compared to the outpatient group (p = 0.033 and p = 0.003). D-dimer levels were significantly higher in the intensive care group compared to the outpatient and hospitalized groups (p = 0.000). Similarly, CRP and ferritin levels increased significantly with disease severity (p = 0.000). Among hematological parameters, leukocyte count showed a significant increase in the intensive care group (p = 0.000), while lymphocyte count decreased (p = 0.000). Neutrophil percentage and neutrophil count were markedly increased in intensive care patients (p = 0.000).

Table 2. Differences in Netosis, Pyroptosis Markers, and Biochemical Parameters Among Outpatient, Hospitalized, and Intensive Care Patient Groups.

	Outpatient Patients (n=50)	Hospitalized Patients (n=50)	Intensive Care Patients (n=50)	p
NE (ng/mL)	757.91 (639.33-900.44) c**	751.98 (615.16-934.56) c**	487.95 (297.23-827.92)a**,b**	0.002
IL-8 (pg/mL)	62.19 (56.29-68.59)	66.75 (56.07-74.55)	65.39 (58.84-79.30)	0.217
GASD-D (ng/mL)	267.59 (215.14-301.04)	271.39 (244.02-321.56)	250.86 (223.12-289.25)	0.117
IL-18 (pg/mL)	25.17 (17.73-42.95) b*	36.79 (23.94-50.58) a*	34.37 (21.84-47.90)	0.033
MPO (ng/mL)	3.12 (1.67-4.21) b**	3.95 (2.47-5.98) a**	3.28 (2.70-4.81)	0.030
CITH3 (ng/mL)	46.40 (40.09-52.31) b**, c**	54.64 (45.10-68.10) a**	57.21 (42.63-76.51) a**	0.003
cfDNA (+ / -)	1/49 (2/98)	1/49	0/50 (0/100)	NA
D-dimer (ng/mL)	0.41 (0.25-0.62) c***	0.52 (0.36-0.74) c***	4.29 (1.17-14.60)a***,b***	0.000

Hemoglobin	13.18 ±1.85	12.96±2.12	12.26±2.12	0.71
Leukocyte (10^3/mcL)	5.86 (5.0-7.0) c***	7.09 (5.47-9.53) c***	11.03 (9.51-14.87) a***,b***	0.000
Lymphocyte (10^3/mcL)	1.5 (1.10-2.10) b**, c***	1.0 (0.7-1.50) a**, c***	0.6 (0.50-0.95)a***,b***	0.000
Platelet (10^3/mcL)	217.49±76.21	257.65±119.38	238.41±116.41	0.184
CRP (mg/L)	33.39±37.52 c***	66.40±59.40 c***	138.23±110.70 a, b*****	0.000
Ferritin	151.80 (58-311.70) b***,c***	492.60 (227.57-793.20) a***, c**	918.30 (567.00-1273.50) b**, a***	0.000
Neutrophil Percentage	65.54±12.77 c***	72.61±14.51 c***	87.92±6.16 a***,b***	0.000
Neutrophil Count (, 0^3/mcL)	3.76 (2.83-5.09) c***	5.12 (3.72-7.01) c***	3.73 (7.42-13.49) a***,b***	0.000

The significance of the differences between groups is indicated by the letters a, b, and c for the outpatient, hospitalized, and intensive care patients groups respectively. * p <0.05, ** p <0.01, *** p < 0.000.

When comparing the demographic characteristics and biochemical parameters of intubated and non-intubated intensive care patients, the mean age of intubated patients (61.22 ± 14.14) was significantly higher than that of non-intubated patients, and this difference was statistically significant (p = 0.027). No significant differences were found between intubated and non-intubated patients for markers such as NE, GASD-D, IL-18, MPO, ferritin, leukocyte count, lymphocyte count, and neutrophil percentage (neu%) (p> 0.05). However, IL-8 and CITH3 levels were significantly higher in intubated patients compared to non-intubated patients (p <0.05). Additionally, CRP levels were significantly higher in intubated patients than in non-intubated patients (p = 0.005). When examining differences among groups based on COVID-19 disease severity, NE and CITH3 markers showed significant differences as infection severity increased. A particularly high level of statistical significance was observed between the outpatient and intensive care groups for these two markers (p <0.05). In contrast, MPO and IL-18 markers showed significant differences mainly between the outpatient and hospitalized groups but were not significantly different between the hospitalized and intensive care groups (Table 2).

DISCUSSION

It is well known that the excessive activation of the immune system and the inflammatory response play a significant role in disease severity and complications in COVID-19, which is caused by SARS-CoV-2 infection. In this study, netosis and pyroptosis markers were analyzed in patient groups with mild, moderate, and severe disease, as well as in a healthy control group. Additionally, the relationship between clinical parameters, disease severity, and these cell death markers was evaluated. In our study, the mean age of COVID-19 patients was significantly higher compared to the healthy control group (mean age: 53.09 vs. 36.63). This finding supports the notion that COVID-19 has more severe effects, particularly on elderly individuals. Specifically, disease severity increased in older age groups (mean age of intensive care patients: 59.36 ± 15.48 years), and male patients had a higher rate of intensive care admission. These findings are consistent with previous studies in the literature that report more severe disease progression in older individuals and males [22].

The presence of comorbidities was observed at rates of 44% in outpatients, 64% in hospitalized patients, and 62% in intensive care patients, but no statistically significant difference was found between the groups. When analyzing the distribution of specific diseases, metabolic diseases, hypertension, respiratory diseases, and cardiovascular diseaseswere more frequent in intensive care patients, but the differences were not statistically significant. COVID-19 patients had a higher prevalence of comorbidities and metabolic diseases (hypertension, respiratory diseases, cardiovascular diseases, renal failure, etc.) compared to the healthy control group. This suggests that COVID-19 may lead to more severe outcomes in individuals with underlying health conditions.

In this study, we examined the impact of disease severity on inflammatory and cell death markers in SARS-CoV-2-infected individuals. When analyzing the differences between groups according to COVID-19 disease severity, NE and CITH3 markers showed significant differences as infection severity increased. A high level of statistical significance was detected for these two markers, particularly between outpatients and intensive care patients. In contrast, MPO and IL-18 markers showed significant differences mainly between outpatients and hospitalized patients, but no significant differences were found between the hospitalized and intensive care groups. These results support the association between biomarkers and infection severity. Other studies have also found that high NE and CITH3 levels are associated with more severe stages of COVID-19 and that these markers correlate significantly with disease severity [23,24].

This study highlights the critical role of inflammatory responses, particularly netosis and pyroptosis processes, in the progression and severity of COVID-19. Inflammatory markers such as ferritin, CRP, and IL-8 were significantly elevated with increasing disease severity. The observation that IL-8 and CitH3 levels were higher in intubated patients suggests that active netosis may contribute to the inflammatory processes underlying severe COVID-19

cases. The significant increase in CRP levels in intubated patients is also an indicator that systemic inflammation is closely associated with disease progression. Furthermore, the significant increase in ferritin levels in the patient group compared to healthy controls suggests a hyperinflammatory response. These findings are consistent with previous studies proposing that pyroptosis and netosis play a role in the pathogenesis of SARS-CoV-2. However, the lack of significant differences in MPO and NE markers between intubated and non-intubated patients suggests that the exact role of these molecules in the inflammatory process of COVID-19 patients remains unclear. Further studies are needed to elucidate the precise mechanisms involved in netotic cell death and inflammation in severe COVID-19 cases.

Our findings indicate a significant increase in NE, GASD-D, and CITH3 levels in COVID-19 patients compared to healthy individuals. This increase suggests enhanced neutrophil activity and pyroptotic cell death, highlighting their association with inflammatory responses. These biomarkers, which play a role in netosis and inflammasome activation, may have both protective and damaging effects. Furthermore, NE levels in intensive care patients were found to be significantly lower, which could indicate suppression or depletion of netosis mechanisms in critical care conditions. This suggests that in advanced stages of COVID-19, excessive NET formation may contribute to microthrombosis and microvascular damage, impairing organ function. Similar to our findings, Yada et al. proposed that NETosis and NET formation play a role in disease progression and thrombosis development in severe and critical COVID-19 cases. Their study found that while NET formation increased in severe COVID-19 cases, NETosis capacity decreased in critical patients [25].

Netosis is a double-edged sword in viral infections, particularly in COVID-19. NETs, composed of chromatin and antimicrobial proteins, help trap and neutralize pathogens. However, excessive NET formation can cause tissue damage, exacerbating inflammation and worsening the hyperinflammatory state observed in severe COVID-19 cases. This aligns with previous studies linking elevated NET levels to disease severity and mortality in COVID-19 patients [26]. Pyroptosis, a cellular defense mechanism against infections or cellular stress, is associated with an inflammatory response. However, in diseases such as COVID-19, excessive activation of this mechanism can intensify inflammation, cause tissue damage, and lead to severe disease progression. The elevated GASD-D levels observed in our patients emphasize the role of pyroptosis in disease pathogenesis. Previous studies have shown that GASD-D activation amplifies the inflammatory response, contributing to cytokine storms and multi-organ damage in severe COVID-19 cases [27, 28].

Differences in IL-8 and CITH3 levels across disease severity groups may be related to intensive care interventions such as intubation. The higher levels of these markers in intubated patients suggest that mechanical ventilation and other invasive procedures may increase their release. Similarly, CRP levels were found to be significantly higher in intubated patients, confirming its role as an inflammatory response marker. The marked elevation of CITH3 levels in intensive care patients, particularly in intubated cases, suggests that severe COVID-19 is associated with excessive histone citrullination. Histone citrullination has been linked to uncontrolled immune responses and tissue damage, which are more pronounced in critically ill patients. Previous research has demonstrated a strong correlation between increased circulating CITH3 levels and disease severity in severe COVID-19 cases [29].

In COVID-19 patients, levels of D-dimer, leukocytes, CRP, neutrophils, and acute-phase reactants such as ferritin were significantly higher than in healthy controls. These findings confirm that COVID-19 triggers strong inflammatory and immunological responses, influencing disease severity and prognosis. Studies by Zhang B et al. and Song CY et al. have also suggested that elevated blood neutrophil levels serve as early indicators of SARS-CoV-2 infection, leading to severe respiratory disease and worse clinical outcomes [30, 31]. Higher D-dimer, CRP, and ferritin levels are clear indicators of hypercoagulability and systemic inflammation in intensive care patients. Increased D-dimer levels have been linked to thromboinflammatory responses in COVID-19, making it a key biomarker for severe disease and mortality risk. These findings align with existing literature emphasizing the association between inflammation, coagulation, and disease severity in COVID-19 [32].

In our study, intensive care patients exhibited higher levels of NE, IL-8, GASD-D, MPO, CITH3, ferritin, D-dimer, leukocytes, lymphocytes, platelets, CRP, neutrophil percentage (neu%), and neutrophil count (neu#) compared to other groups. Similarly, higher biomarker levels were observed in intubated and deceased patients, confirming that SARS-CoV-2-induced COVID-19 can lead to severe complications such as respiratory failure and fatal outcomes. A study by Çelik M et al. reported that GASD-D, caspase-1, IL-1β, NLRP3, CRP, and ferritin levels were significantly elevated in COVID-19 patients compared to controls. Similarly, our findings indicate that inflammatory markers such as GASD-D, CRP, and ferritin are increased in COVID-19 patients and correlate with disease severity, further reinforcing the role of inflammatory responses in disease progression [33]. Our findings reveal a strong correlation between circulating inflammatory markers and COVID-19 severity, providing crucial insights for developing COVID-19 treatment strategies. Targeting netosis and pyroptosis may offer therapeutic potential to reduce disease severity and prevent related complications. These approaches could modulate the immune response and suppress excessive inflammation, ultimately improving clinical outcomes in

COVID-19 patients. Future therapeutic strategies aimed at controlling NET formation and pyroptosis may help mitigate excessive inflammatory responses and improve survival rates in severe COVID-19 cases.

This study has some limitations. The patient population was recruited from a single center, and the sample size was not expanded, which may limit the generalizability of the findings. Future studies with larger and more diverse patient populations will provide stronger evidence on the role of netosis and pyroptosis markers in COVID-19 pathophysiology. Our study underscores the critical role of inflammatory responses, particularly netosis and pyroptosis, in COVID-19 pathogenesis. The observed increases in IL-8, GSDMD, and CITH3 levels in our patient group suggest that dysregulated immune responses contribute to disease severity and poor clinical outcomes. The potential clinical utility of these biomarkers should be further evaluated in larger-scale studies to determine their role in disease management and prognosis.

CONCLUSION

In this study, we investigated the role of netosis and pyroptosis in COVID-19 pathogenesis by analyzing inflammatory and cell death markers in patients with varying disease severity. Our findings demonstrate a strong correlation between disease severity and elevated levels of key biomarkers such as NE, IL-8, GSDMD, MPO, CITH3, ferritin, D-dimer, and CRP. Notably, the increased levels of IL-8 and CITH3 in intubated patients suggest that active netosis may contribute to the excessive inflammatory response observed in severe COVID-19 cases. Similarly, the elevated GASD-D levels highlight the role of pyroptosis in driving hyperinflammatory responses, potentially leading to cytokine storms and multi-organ damage.

The significant increase in inflammatory markers, particularly ferritin, CRP, and IL-8, reinforces the link between immune dysregulation and COVID-19 severity. Our results also suggest that excessive NET formation may contribute to microvascular damage and thrombosis, impairing organ function in critically ill patients. The observation that NE levels were lower in intensive care patients raises the possibility that netosis mechanisms may become suppressed in advanced disease stages, possibly due to neutrophil depletion or immune exhaustion.

Overall, our study underscores the importance of targeting netosis and pyroptosis as potential therapeutic strategies for severe COVID-19. Modulating these inflammatory pathways could help control excessive immune activation, reduce systemic inflammation, and improve clinical outcomes. However, further research is needed to fully elucidate the mechanistic links between netosis, pyroptosis, and COVID-19 severity. Larger-scale studies with diverse patient populations will be crucial in validating the clinical utility of these biomarkers and exploring targeted therapeutic approaches aimed at mitigating inflammatory complications in COVID-19 patients.

Conflict Of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. No financial support or benefits have been received by any of the authors, nor do any of the authors have personal relationships that could inappropriately influence (bias) this work.

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Annotation

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