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Predictive Value of dsDNA and Nucleosomes as Neutrophil Extracellular Traps-Related Biomarkers for COVID-19 in Older Patients

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Background: Previous studies have demonstrated that neutrophil extracellular traps (NETs) are crucial in infectious diseases. This study aims to evaluate the clinical value of NET-related biomarkers in identifying the risk of COVID-19 and diagnosing the disease.
Methods: This study involved 32 patients who tested positive for COVID-19 via polymerase chain reaction (PCR) between April and August 2023. During the same period, 30 healthy volunteers were enrolled as a control group. The principal biomarkers related to NETs are citrullinated histone H3 (CitH3), double-stranded DNA (dsDNA), myeloperoxidase-DNA complex (MPO-DNA), and Nucleosome. Elevated levels in two or more of these biomarkers indicate raised NET concentrations. Multivariable logistic regression analysis was employed to assess whether NET-related biomarkers were the independent risk factor of COVID-19. The diagnostic value of NET-related biomarkers in COVID-19 was further evaluated using receiver operating characteristic (ROC) curve analysis. Statistical procedures were executed in SPSS software (version 24.0, USA).
Results: Compared with the control group, patients infected with COVID-19 had higher levels of dsDNA and nucleosomes ($P < 0.001$). Correlation analysis revealed a positive correlation between dsDNA levels and neutrophil count ($r = 0.309$, $P = 0.015$) as well as between nucleosome levels and neutrophil count ($r = 0.446$, $P < 0.001$). Further analysis showed that dsDNA and nucleosomes were independent risk factors for COVID-19 infection. ROC curve analysis showed that dsDNA area under the curve (AUC) = 0.777, 95% confidence interval (CI), 0.661–0.893, $P < 0.001$, and nucleosomes (AUC = 0.884, 95% CI, 0.778–0.991, $P < 0.001$) had well diagnostic value in the diagnosing COVID-19 infection.
Conclusion: NET-related biomarkers, dsDNA and nucleosomes, were independent risk factors of COVID-19 infection and potentially useful biomarkers in diagnosing COVID-19 infection in older patients.
Keywords: neutrophil extracellular traps, COVID-19, pneumonia, dsDNA, nucleosomes, older patient

Introduction

The coronavirus disease 2019 (COVID-19) is an acute respiratory infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which seriously threatens human life.[1] Between January 1, 2020, and December 31, 2021, there were 5.94 million reported COVID-19 deaths worldwide.[2,3] In the context of universal vaccination against COVID-19, the global population remains particularly susceptible to infection by SARS-CoV-2, the pathogen responsible for COVID-19.[4] In particular, elderly patients, due to the decline of immune system function and various complications, are prone to be infected by SARS-CoV-2, causing COVID-19. Elderly patients with COVID-19 are prone to serious complications and high mortality.

Although polymerase chain reaction (PCR) testing is currently the gold standard for diagnosing COVID-19, these tests often face reporting delays and may occasionally fail to detect the virus. Therefore, there is an urgent need to

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identify new biomarkers that can facilitate early and accurate diagnosis of COVID-19 and compensate for the limitations of PCR technology, ultimately providing a more comprehensive assessment of a patient's infection status and disease progression at different levels.[5,6]

Neutrophil Extracellular Traps (NETs) are a network of structures released by neutrophils to trap and kill pathogens in response to infection. This web-like structure is primarily composed of extracellular DNA serving as a scaffold, embedded with various active proteins, including neutrophil elastase (NE), cathepsin G (Cath G), and myeloperoxidase (MPO).[7] NETs form a defensive barrier through their adhesion and the action of reactive oxygen species (ROS), which can capture and invade pathogens at the site of infection and effectively destroy the captured pathogens, thus protecting the body from infection.[8] Studies have confirmed that neutrophil extracellular traps (NETs) play a crucial role in the containment and clearance of Staphylococcus aureus and Streptococcus pneumoniae.[9,10] These bacteria are known for their virulence and potential to cause severe infections, and NET formation is often effective in neutralizing them. However, excessive or abnormal formation of NETs can also lead to self-tissue damage and has been associated with a variety of inflammatory and autoimmune diseases.[11–13] Recent research has revealed that histones released by NETs enhance the infectivity of SARS-CoV-2 by connecting the virus's spike protein subunit 2 with sialic acid on host cells and play a significant role in contributing to immune-thrombosis and necroinflammation in patients suffering from COVID-19.[14,15]

Thus, this study intends to comprehensively evaluate the clinical value of the above four NET-related biomarkers in the risk prediction and diagnosis of elderly patients with COVID-19.

Materials and Methods

Study Design and Population

This cross-sectional study was conducted at Inner Mongolia Baogang Hospital in Baotou, China. From April to August 2023, 32 COVID-19 patients and 30 healthy volunteers who underwent physical examinations in our hospital enrolled. The inclusion criteria for this study are as follows: (1) Patients who had a confirmed diagnosis of COVID-19 and aged over 60 years old. (2) A control group consisting of individuals over 60 years old, with gender matching as closely as possible, and including complete clinical and laboratory data for this study. Exclusion criteria ruled out individuals with a history of liver disease, hematological disorders, malignancies, potential comorbidities such as AIDS or other immunocompromised conditions, as well as those who refused to provide informed consent.

The study protocol complied with the Declaration of Helsinki and has been officially approved by the Ethics Review Committee of Inner Mongolia Baogang Hospital. All procedures implemented during the study followed routine clinical practice to ensure minimal interference with participants' standard medical protocols. Meanwhile, the collected data is anonymous to protect the privacy of participants and ensure compliance with the ethical standards of the study. All participants in this study have signed informed consent.

Clinical Definition

The diagnosis of COVID-19 should be based on a thorough analysis of the individual's epidemiological history, clinical symptoms, and laboratory test results. The primary criterion for diagnosis is a positive nucleic acid test for the novel coronavirus. The diagnostic criteria for COVID-19 include:

1) Clinical symptoms related to COVID-19 infection;

2) Having one or more of the following etiological and serological test results:

- Positive COVID-19 nucleic acid test.
- Positive test for COVID-19 antigen.
- Positive COVID-19 isolation and culture.

In this study, the diagnostic criterion for COVID-19 is a positive result from either nucleic acid testing or antigen detection for the novel coronavirus. The diagnosis of COVID-19 was made by two study investigators.

Data Collection

The demographic and laboratory data at admission were collected from electronic medical records, including age, gender, body temperature, respiratory rate, heart rate, and the levels of white blood cell (WBC), hemoglobin (Hb), platelet (PLT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA), blood urea nitrogen (Urea), and creatinine (CREA). White blood cell count, neutrophil count, and platelet count analyses were measured using the manufactured Maccura F81 fully automated blood analyzer (Maccura Biotechnology, Sichuan, China). Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA), urea nitrogen (UREA), and creatinine (Cr) were quantified using the Siemens 2400 fully automated biochemical analyzer (Siemens Healthineers, Erlangen).

Measurement of NET-Related Biomarkers

The assessment of NET-related biomarkers, including citrullinated histone H3 (CitH3), double-stranded DNA (dsDNA), myeloperoxidase-DNA complex (MPO-DNA), and nucleosome, were assessed by examining plasma components. The presence of circulating citrullinated histone H3 was ascertained using an ELISA kit (Cayman Chemical). The plasma dsDNA levels were determined utilizing the Quant-iT™ PicoGreen® dsDNA Reagent and Kits (Invitrogen, Cat. #P7589). The levels of MPO-DNA complexes were determined via a modified enzyme-linked immunosorbent assay (ELISA) technique. Circulating nucleosomes were quantified using an ELISA kit (Cell Death Detection ELISA PLUS, Roche Diagnostics, IN, USA) with adapted manufacturer instructions.[16]

Statistical Analysis

Data conforming to a normal distribution were analyzed using *t*-tests or one-way ANOVA, with results presented as mean ± standard deviation (SD). Conversely, for data not adhering to a normal distribution, medians (interquartile range) were reported, and analyses were conducted employing the Mann–Whitney *U*-test. Additionally, categorical data were quantified as percentages and assessed using the chi-square test for statistical evaluation. The connection between dsDNA, nucleosome levels, and other continuous measures was inspected using Spearman's rank correlation test. To pinpoint independent predictors of COVID-19, a multivariable logistic regression analysis was carried out, including variables with P-values below 0.05 from the univariate analysis in the multivariable model. Additionally, receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic effectiveness of dsDNA and Nucleosome levels for COVID-19, with the area under the curve (AUC) assessed through DeLong's test. The Youden Index helped find the best cutoff point. A two-sided p-value less than 0.05 is often conventionally regarded as statistically significant.

Results

Study Population Characteristics

A total of 62 subjects were recruited in this study. Among them, 32 individuals were diagnosed with COVID-19 and defined as the COVID-19 group and 30 healthy volunteers acted as the control group. The basic characteristics of the study population are presented in Table 1. Compared with the control group, patients with COVID-19 were older and displayed higher respiratory rates and heart rates. Biochemical and hematologic parameters analysis showed that the level of ALT, AST and neutrophil counts were significantly higher in patients with COVID-19. Further analysis showed that patients with COVID-19 had a lower level of UA and PLTs. Meanwhile, we examined the NET-related biomarkers between the two groups. As presented in Table 2, patients with COVID-19 had significantly higher levels of CitH3, dsDNA, and Nucleosome compared to the control group, which indicated that patients with COVID-19 had a higher level of NETs.

Correlation Between NET-Related Biomarkers and Clinical Parameters

As shown in Table 3, CitH3 shows positive correlations with temperature (r = 0.254, P = 0.047), neutrophil count (r = 0.264, P = 0.038), ALT (r = 0.269, P = 0.034), and a negative correlation with PLT (r = −0.254, P = 0.046). The dsDNA positively correlated with age (r = 0.322, P = 0.011) and neutrophil count (r = 0.309, P = 0.015). Nucleosome exhibits positive correlations with age (r = 0.322, P = 0.011), respiratory rate (r = 0.264, P = 0.038), heart rate (r = 0.401,

Table 1 Basic Characteristics of Study Subjects

Variables	Control (n=30)	COVID-19 (n=32)	P
Age (year)	68.50 (66.75, 73.00)	76.50 (68.50, 82.00)	0.002
Male, n (%)	15.00 (50.00%)	18.00 (56.30%)	0.313
Temperature (°C)	36.55 (36.475, 36.75)	36.70 (36.30, 37.70)	0.245
Respiratory (rate/minute)	20.00 (18.00, 21.00)	21.00 (20.00, 21.00)	0.012
Heart rate (rate/minute)	79.00 (72.75, 83.25)	83.50 (76.00, 97.00)	0.012
Hypertension, n (%)	11.00 (40.00%)	18.00 (56.25%)	0.204
Diabetes mellitus, n (%)	2.00 (6.67%)	8.00 (25.00%)	0.052
Heart disease, n (%)	9.00 (30.00%)	7.00 (21.88%)	0.469
Biochemical parameters			
WBC ($\times 10^9$cell/L)	5.86 (4.82, 7.60)	6.09 (4.90, 7.74)	0.486
Neutrophil ($\times 10^9$cell/L)	2.93 (2.38, 4.19)	5.19 (3.66, 6.60)	< 0.001
PLT ($\times 10^9$cell/L)	222.90±49.60	185.47±78.10	0.029
ALT (U/L)	21.50 (17.00, 28.00)	29.00 (19.25, 44.50)	0.027
AST (U/L)	21.00 (18.75, 23.50)	29.50 (20.00, 41.75)	0.001
UA (mmol/L)	301.50 (274.75, 375.50)	226.00 (166.25, 268.25)	< 0.001
BUN (umol/L)	5.55 (4.38, 6.23)	6.25 (5.03, 7.58)	0.101
CREA (umol/L)	71.7 (56.65, 80.03)	72.85 (54.18, 84.03)	0.800
Mortality, n (%)	0.00 (00.00%)	7 (21.88%)	0.007

Abbreviations: COVID-19, corona virus disease 2019; WBC, white blood cell; Hb, hemoglobin; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; UA, uric acid; Urea, blood urea nitrogen; CREA, creatinine.

Table 2 NET-Related Biomarkers Levels Between the Two Groups

Variables	Control (n=30)	COVID-19 (n=32)	P
CitH3 (ng/mL)	1.728 (1.454, 2.369)	2.123 (1.392, 4.025)	0.0269
dsDNA (10^{-1}ug/mL)	4.770 (4.470, 5.360)	6.190 (5.110, 7.180)	< 0.001
MPO-DNA (Fold change over control)	0.673 (0.497, 1.334)	0.478 (0.234, 0.912)	0.099
Nucleosome (Fold change over control)	0.749 (0.419, 1.187)	1.850 (1.528, 2.146)	< 0.001

Abbreviations: COVID-19, coronavirus disease 2019; CitH3, citrullinated histone H3; dsDNA, double-stranded DNA; MPO-DNA, myeloperoxidase-DNA complex.

Table 3 Correlation Between NET-Related Biomarkers and Clinical and Laboratory Indexes

Variables	CitH3		dsDNA		MPO-DNA		Nucleosome	
	r	P	r	P	r	P	r	P
Age (year)	0.044	0.734	0.322	0.011	−0.073	0.571	0.322	0.011
Temperature (°C)	0.254	0.047	0.219	0.088	−0.064	0.623	0.219	0.087
Respiratory (rate/minute)	0.204	0.112	0.179	0.164	−0.200	0.119	0.264	0.038
Heart rate (rate/minute)	0.231	0.071	0.210	0.101	0.173	0.178	0.401	0.001
Neutrophil ($\times 10^9$cell/L)	0.264	0.038	0.309	0.015	−0.014	0.917	0.446	<0.001
PLT ($\times 10^9$cell/L)	−0.254	0.046	−0.208	0.105	−0.080	0.538	0.316	0.012
ALT (U/L)	0.269	0.034	0.059	0.649	−0.006	0.962	0.273	0.032
AST (U/L)	0.154	0.233	0.186	0.148	0.076	0.558	0.343	0.006
UA (mmol/L)	−0.041	0.754	−0.163	0.204	0.255	0.045	0.424	0.001

Abbreviations: PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; UA, uric acid; CitH3, citrullinated histone H3; dsDNA, double-stranded DNA; MPO-DNA, myeloperoxidase-DNA complex.

Table 4 Predictive Value of NET-Related Biomarkers for COVID-19

Variables	Univariate		Multivariate*	
	OR (95% CI)	P	OR (95% CI)	P
CitH3 (ng/mL)	1.252 (0.979–1.600)	0.073	—	—
dsDNA (10^{-1}ug/mL)	2.290 (1.294–4.053)	0.004	1.881 (1.007–3.514)	0.048
MPO-DNA (Fold change over control)	0.994 (0.601–1.643)	0.981	—	—
Nucleosome (Fold change over control)	7.195 (2.468–20.978)	<0.001	3.009 (1.052–8.607)	0.040

Notes: *Adjusted for age, temperature, respiratory rate, heart rate, neutrophils, ALT, and AST.
Abbreviations: CI, confidence interval; CitH3, citrullinated histone H3; dsDNA, double-stranded DNA; MPO-DNA, myeloperoxidase-DNA complex; CI, confidence interval.

Table 5 AUC of NET-Related Biomarkers in Identifying COVID-19

Variables	AUC	95% CI	Sensitivity	Specificity	P
CitH3 (ng/mL)	0.582	0.436–0.727	37.5%	90.0%	0.269
dsDNA (10^{-1} ug/mL)	0.777	0.661–0.893	62.5%	83.3%	<0.001
MPO-DNA (Fold change over control)	0.378	0.234–0.522	21.9%	93.3%	0.099
Nucleosome (Fold change over control)	0.884	0.778–0.991	96.9%	86.7%	<0.001

Abbreviations: AUC, area under the ROC curve; CI, confidence interval; CitH3, citrullinated histone H3; dsDNA, double-stranded DNA; MPO-DNA, myeloperoxidase-DNA complex.

P = 0.001), neutrophil count (r = 0.446, P < 0.001), PLT (r = 0.316, P = 0.012), ALT (r = 0.273, P = 0.032), AST (r = 0.343, P = 0.006), and UA (r = 0.424, P = 0.001) levels. The correlation between MPO-DNA and UA is statistically significant (r = 0.255, P = 0.045) with a positive association.

Predictive Value NET-Related Biomarkers for COVID-19

Multivariable logistic regression analysis was used to test whether these NET-related biomarkers were independent risk predictors for COVID-19. Indicators with P-values less than 0.05 in the univariate regression analysis were included in multivariable regression analysis, which included age, temperature, respiratory rate, heart rate, neutrophil count, ALT, and AST. After adjusting the above factors, dsDNA (OR = 1.881, 95% confidence interval (CI) = 1.0007–3.514, P = 0.048) and Nucleosome (OR = 3.009, 95% CI = 1.052–8.607, P = 0.040) remained independent predictors for COVID-19 (Table 4).

Diagnostic Value of NET-Related Biomarkers in COVID-19

ROC curve analysis assessed the diagnostic efficacy of NET-related biomarkers for COVID-19. As depicted in Table 5, the AUC for CitH3, dsDNA, MPO-DNA and Nucleosome predicting overall COVID-19 diagnosis was 0.582 (95% CI: 0.436–0.727, P = 0.269), 0.777 (95% CI: 0.661–0.893, P < 0.001), 0.378 (95% CI: 0.234–0.522, P = 0.099), and 0.884 (95% CI: 0.778–0.991, P < 0.001). The optimal cutoff values for CitH3, dsDNA, MPO-DNA, and Nucleosome were 3.144 (ng/mL), 5.767 (10^{-1}ug/mL), 2.456 (Fold change over control), and 1.314 (Fold change over control).

Discussion

COVID-19 is a severe respiratory illness caused by the novel SARS-CoV-2 virus. This virus presents a significant global health risk, especially for older individuals. It is primarily transmitted through respiratory droplets released when an infected person coughs, sneezes, or talks. Additionally, it can spread through contact with contaminated surfaces and subsequent touching of the face. The symptoms of COVID-19 can range from mild to severe, with common manifestations including fever, dry cough, and fatigue. In severe cases, the illness can progress to pneumonia, acute respiratory distress syndrome (ARDS), multi-organ failure, and even death.

NETs were first discovered by Brinkmann et al when neutrophils are stimulated by lipopolysaccharide (LPS) or interleukin-8 (IL-8); neutrophils generate a unique extracellular structure comprising a fibrous network. The principal biomarkers related to NETs are CitH3, dsDNA, MPO-DNA, and Nucleosome, which are identified by peripheral blood.

Elevated levels in two or more of these biomarkers indicate raised NET concentrations. Recent research has shown that the role of NETs in various diseases, including infectious diseases and autoimmune diseases, has received increasing attention.[17–19] In cases of community-acquired pneumonia (CAP), patients with elevated serum NET-related biomarkers face increased risks of clinical instability, prolonged hospitalization, and higher all-cause mortality within 30 days.[20] Individuals in the advanced stages of Chronic Obstructive Pulmonary Disease (COPD) often have a significantly higher concentration of NETs. This increase is associated with a higher frequency of exacerbations and a simultaneous decrease in microbiota diversity.[21] Furthermore, recent studies have shown that administering long-acting DNase-1, which consists of DNase-1-coated polydopamine-poly nanoparticles via the intravenous route, can inhibit NET-related biomarkers in blood samples to enhance survival rates in models of sepsis. By detecting biomarkers related to NETs, the body's inflammatory response and immune status can be assessed.[22,23] Hayder et al revealed that excessive development of NETs in SARS-CoV-2 infection is linked with the development of ARDS.[24,25] However, whether NETs can diagnose COVID-19 remains unknown.

In this study, we explored the association between NET-related biomarkers and COVID-19 in older patients. We found that the CitH3, dsDNA, and Nucleosome were significantly higher in COVID-19 patients by the control group, which indicated that the level of NETs increased in COVID-19 patients Unlike previous studies that primarily focused on cytokine storms in COVID-19 infections,[26] our investigation provides a unique perspective by integrating the role of NETs and their biomarkers in elderly patients. Although a recent study by de Buhr et al[27] examined NETs in elderly COVID-19 patients, it did not address the relationship between clinical indicators and NET biomarkers. In contrast, our research expands the sample size, targets an under-researched population in China, and demonstrates that NET-related biomarkers, including CitH3, dsDNA, and nucleosomes, are significantly and positively correlated with neutrophil count, an established marker of inflammation. Multivariable analysis has highlighted NET-related biomarker levels as an independent risk for the infection of COVID-19, even after adjusting for other known risk factors. Other non-significant correlations may be constrained by the sample size and require validation in larger, more diverse populations to fully understand their scope and reliability. Confounding variables like coexisting diseases or inflammation may also influence the relationship between NET-related biomarkers and measures such as ALT and AST. Therefore, further investigation is needed to clarify this complex association.

Our study has several limitations. First, the small sample size restricts the generalizability of the findings, highlighting the need for replication in larger, more diverse cohorts. Second, the absence of longitudinal data impedes our ability to establish temporal dynamics and causal links. Finally, due to the lack of standardization, the use of fold change for quantification limits the comparability of results across studies.

Conclusions

This study found that levels of both dsDNA and nucleosomes were significantly increased in elderly patients with COVID-19 compared to the control group. These biomarkers serve as independent risk factors, enhancing the ability to identify individuals at high risk for COVID-19. Furthermore, the elevated levels of dsDNA and nucleosomes correlate strongly with disease severity, indicating their potential as reliable diagnostic indicators for early detection and prognosis of COVID-19.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics Statement

The study was conducted according to the Declaration of Helsinki policies and received approval from the Hospital Ethics Review Board of Inner Mongolia Baogang Hospital. Informed consent was obtained from all the participants.

Author Contributions

All authors significantly contributed to the work reported in various ways, including the conception and design of the study, its execution, data acquisition, analysis, and interpretation. They participated in drafting, revising, and critically

reviewing the article. Each author provided final approval for the version to be published, agreed on the journal to which the article was submitted, and accepted accountability for all aspects of the work.

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Disclosure

No conflict of interest was declared.

References

1. Li G, De Clercq E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). *Nat Rev Drug Discov*. 2020;19(3):149–150. doi:10.1038/d41573-020-00016-0
2. Wang H, Paulson KR, Pease SA. COVID-19 excess mortality collaborators. Estimating excess mortality due to the COVID-19 pandemic: a systematic analysis of COVID-19-related mortality, 2020-21. *Lancet*. 2022;399(10334):1513–1536. doi:10.1016/S0140-6736(21)02796-3
3. GBD. 2021 Demographics Collaborators. Global age-sex-specific mortality, life expectancy, and population estimates in 204 countries and territories and 811 subnational locations, 1950-2021, and the impact of the COVID-19 pandemic: a comprehensive demographic analysis for the global burden of disease study 2021. *Lancet*. 2024;403(10440):1989–2056. doi:10.1016/S0140-6736(24)00476-8
4. Nordström P, Ballin M, Nordström A. Risk of infection, hospitalisation, and death up to 9 months after a second dose of COVID-19 vaccine: a retrospective, total population cohort study in Sweden. *Lancet*. 2022;399(10327):814–823. doi:10.1016/S0140-6736(22)00089-7
5. Prevention of SARS-CoV-2. COVID-19 treatment guidelines. Available from: https://www.covid19treatmentguidelines.nih.gov/overview/prevention-of-sars-cov-2/. Accessed July 25, 2024.
6. Miller TE, Garcia Beltran WF, Bard AZ, et al. Clinical sensitivity and interpretation of PCR and serological COVID-19 diagnostics for patients presenting to the hospital. *FASEB J*. 2020;34(10):13877–13884. doi:10.1096/fj.202001700RR
7. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science*. 2004;303(5663):1532–1535. doi:10.1126/science.1092385
8. Yang H, Biermann MH, Brauner JM, Liu Y, Zhao Y, Herrmann M. New insights into neutrophil extracellular traps: mechanisms of formation and role in inflammation. *Front Immunol*. 2016;7:302. doi:10.3389/fimmu.2016.00302
9. Fuchs TA, Abed U, Goosmann C, et al. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol*. 2007;176(2):231–241. doi:10.1083/jcb.200606027
10. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J Cell Biol*. 2010;191(3):677–691. doi:10.1083/jcb.201006052
11. Radermecker C, Detrembleur N, Guiot J, et al. Neutrophil extracellular traps infiltrate the lung airway, interstitial, and vascular compartments in severe COVID-19. *J Exp Med*. 2020;217(12):e20201012. doi:10.1084/jem.20201012
12. Veras FP, Pontelli MC, Silva CM, et al. SARS-CoV-2-triggered neutrophil extracellular traps mediate COVID-19 pathology. *J Exp Med*. 2020;217(12):e20201129. doi:10.1084/jem.20201129
13. Tomar B, Anders HJ, Desai J, Mulay SR. Neutrophils and neutrophil extracellular traps drive necroinflammation in COVID-19. *Cells*. 2020;9(6):1383. doi:10.3390/cells9061383
14. Hong W, Yang J, Zou J, et al. Histones released by NETosis enhance the infectivity of SARS-CoV-2 by bridging the spike protein subunit 2 and sialic acid on host cells. *Cell Mol Immunol*. 2022;19(5):577–587. doi:10.1038/s41423-022-00845-6
15. Middleton EA, He XY, Denorme F, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. *Blood*. 2020;136(10):1169–1179. doi:10.1182/blood.2020007008
16. Li T, Peng R, Wang F, et al. Lysophosphatidic acid promotes thrombus stability by inducing rapid formation of neutrophil extracellular traps: a new mechanism of thrombosis. *J Thromb Haemost*. 2020;18(8):1952–1964. doi:10.1111/jth.14839
17. Brinkmann V, Zychlinsky A. Beneficial suicide: why neutrophils die to make NETs. *Nat Rev Microbiol*. 2007;5(8):577–582. doi:10.1038/nrmicro1710
18. Lood C, Blanco LP, Purmalek MM, et al. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nat Med*. 2016;22(2):146–153. doi:10.1038/nm.4027
19. Guo G, Liu Z, Yu J, et al. Neutrophil function conversion driven by immune switchpoint regulator against diabetes-related biofilm infections. *Adv Mater*. 2024;36(8):e2310320. doi:10.1002/adma.202310320
20. Ebrahimi F, Giaglis S, Hahn S, et al. Markers of neutrophil extracellular traps predict adverse outcome in community-acquired pneumonia: secondary analysis of a randomised controlled trial. *Eur Respir J*. 2018;51(4):1701389. doi:10.1183/13993003.01389-2017
21. Dicker AJ, Crichton ML, Pumphrey EG, et al. Neutrophil extracellular traps are associated with disease severity and microbiota diversity in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol*. 2018;141(1):117–127. doi:10.1016/j.jaci.2017.04.022
22. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, et al. Targeting potential drivers of COVID-19: neutrophil extracellular traps. *J Exp Med*. 2020;217(6):e20200652. doi:10.1084/jem.20200652

23. Skendros P, Mitsios A, Chrysanthopoulou A, et al. Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. *J Clin Invest*. 2020;130(11):6151–6157. doi:10.1172/JCI141374
24. Al-Kuraishy HM, Al-Gareeb AI, Al-Hussaniy HA, Al-Harcan NAH, Alexiou A, Batiha GES. Neutrophil extracellular traps (NETs) and Covid-19: a new frontiers for therapeutic modality. *Int Immunopharmacol*. 2022;104:108516. doi:10.1016/j.intimp.2021.108516
25. Zhang R, Sun C, Han Y, et al. Neutrophil autophagy and NETosis in COVID-19: perspectives. *Autophagy*. 2023;19(3):758–767. doi:10.1080/15548627.2022.2099206
26. Bhaskar S, Sinha A, Banach M, et al. Cytokine storm in COVID-19-immunopathological mechanisms, clinical considerations, and therapeutic approaches: the REPROGRAM consortium position paper. *Front Immunol*. 2020;11:1648. doi:10.3389/fimmu.2020.01648
27. de B N, Ac P, S M, et al. Impaired degradation of neutrophil extracellular traps: a possible severity factor of elderly male COVID-19 patients. *J. Innate Immun*. 2022;14(5). doi:10.1159/000521594