Neutrophil-Lymphocyte Ratio Predicting Case Severity in SARS-CoV-2 Infection: A Review

Sonal Agarwal [1]

1. Department of Pathology, Mahatma Gandhi Hospital, Rajmata Vijaya Raje Scindia Medical College, Bhilwara, IND

Corresponding author: Sonal Agarwal, jsonal77@gmail.com

Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is highly contagious and has taken an enormous toll on the worldwide quality of life and the global economy, in addition to the lives lost due to coronavirus disease 2019 (COVID-19). Precautionary measures and timely identification of the infected cases are essential to minimize the spread of SARS-CoV-2. Infection with this virus causes a spike in the proinflammatory cytokines, resulting in immune system-mediated host tissue damage, thus leading to mortality. Therefore, identifying mild, moderate, and severe cases is crucial to rendering appropriate care. Recent research has focused on identifying laboratory techniques to predict the case severity and outcome of COVID-19 cases. Low serum lymphocyte levels, low lymphocyte-to-C-reactive protein ratio, low platelet-to-lymphocyte ratio, thrombocytopenia, and high neutrophil-lymphocyte ratio (NLR) have been observed in critical infections. NLR might be a prognostic marker for disease severity. Severe cases can be triaged at hospital admission for proper treatment planning and to reduce mortality. This review highlights the potential role of NLR hematological assay in SARS-CoV-2 infection and the mechanism of neutrophilic-induced host tissue damage.

Introduction And Background

The beginning of the 20th century brought three new devastating coronaviruses that affected the entire world. The emergence of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, Middle East respiratory syndrome-related coronavirus in 2012, and SARS-CoV-2 in 2019 led to influenza-like symptoms in humans with severe fatal outcomes [1]. Reportedly originating from bats, the coronaviruses transmitted to intermediate animal hosts [2]. With repeated replication, new variants emerged that were capable of transmission to humans [3]. Transmission of coronavirus disease 2019 (COVID-19), the disease caused by SARS-CoV-2, predominantly occurred in a nosocomial manner by droplet infection or close personal contact. Initial clinical presentation was fever and dry cough that could progress to unilateral/bilateral pneumonia, severe acute respiratory distress syndrome (ARDS), acute cardiac injury, renal failure, multiple organ failure, and death [4]. Reverse transcriptase-polymerase chain reaction confirmed the diagnosis, followed by high-resonance computed tomography (CT) of the chest. Various hematocytometric investigations can reveal high neutrophils, low lymphocyte levels, and high platelet levels in SARS-CoV-2 infections. Cao et al. noted low serum lymphocyte levels in COVID-19 cases [5]. Low lymphocyte-to-C-reactive protein ratio, low platelet-to-lymphocyte ratio, and thrombocytopenia have been observed in critical SARS-CoV-2 infections [6,7]. Patients with severe COVID-19 cases had a higher neutrophil-lymphocyte ratio (NLR) ratio than the milder cases, suggesting that NLR might be a prognostic marker for assessing the disease severity [8]. Thus, severe cases could be identified at the time of hospital admission and triaged for proper treatment planning and reduction in mortality.

Studies report cytokine storm and immune dysfunction in COVID-19 cases, with lower lymphocyte and helper T cell counts [9]. There is a slow rise in lymphocytes during recovery [10]. Thus, lymphocytes have limited use in evaluating COVID-19 severity. However, NLR consistently rises early in the disease course, making it a valuable metric. Various preliminary studies have investigated the role of NLR in assessing the severity of other diseases such as asthma [11,12]. This review highlights the potential role of NLR hematological assay in SARS-CoV-2 infections to determine COVID-19 severity and the mechanism of neutrophilic-induced host tissue damage [12].

Review

Neutrophils in health and disease

Earlier it was thought that neutrophils are a homogenous population with a short life cycle. Lately, heterogeneous populations of neutrophils have been described in infections, autoimmune diseases, and cancers [13]. Pro-neutrophils differentiate into lineage-committed precursors, immature and mature neutrophils. Three distinct subsets of neutrophils have been identified (i.e., homeostatic, aged, and

interferon-stimulated gene-related neutrophils) [14,15]. With the expression of C-X-C motif chemokine receptor 2 (CXCR2), mature neutrophils migrate from the bone marrow into blood vessels [16]. Peak neutrophil sequestration occurs at night following a circadian rhythm. With the upregulation of C-X-C chemokine receptor 4 (CXCR4) chemokine expression, aged neutrophils migrate back to the spleen, bone marrow, or liver, where they are phagocytosed [17].

Being a part of innate immunity, neutrophils function as the first line of defense. Abnormalities in immune regulation can lead to extensive host tissue damage by these granulocytes. They phagocytose bacteria and kill them by fusing with cytoplasmic granules (i.e., oxidative burst). The cytoplasmic granules contain proteases, defensins, antimicrobial peptides, and reactive oxygen species. The chemokine expression of C-X-C motif chemokine ligand (CXCL) 1, 2, and 8 guides neutrophils' migration and activation. Other inflammatory components such as complement 5a and activated platelets cause the localization of neutrophils and the formation of neutrophil extracellular traps (NETs) [17]. NETs are web-like structures of deoxyribonucleic acid (DNA) and proteins expelled from the neutrophil that ensnare pathogens. The neutrophil platelet aggregation leads to a cascade of events, from the formation of fibrin mesh to trapping the pathogens and NET-induced killing in associated areas [18].

Neutrophils and thrombi formation

Increased vascular permeability leads to proteinaceous exudates in alveolar spaces and pulmonary edema in severe inflammation. Proteinase 3 disrupts the tight junctions, and neutrophil seepage occurs through endothelial cells. Mechanisms of NET formation are not fully understood. Some potentially important enzymes involved in forming these extracellular traps are neutrophil elastase, peptidyl arginine deiminases (PAD), and gasdermin D [19]. PADs are essential in the formation of NETs as they are expressed in granulocytes and mediate histone citrullination. Thus, chromatin decondensation takes place and chromosomal DNA is expulsed with various antimicrobial peptides triggering the NET formation. Excessive exaggeration of these traps can trigger a cascade of inflammatory events that cause collateral damage to the host, formation of microthrombi, irreversible lung damage, and cardiac and renal tissues, apart from having beneficial effects [20]. Lung, heart, and kidney tissue are targeted by SARS-CoV-2 and play an important role in COVID-19 mortality [21].

NET disrupts the granules, releasing histones (present in NET chromatin) and granule proteins such as myeloperoxidase, neutrophil elastase, and proteinase, denoting the epithelial lining and causes platelet aggregation and thrombosis [22]. Elevated levels of extracellular histones have been found in the extracellular lavage and plasma of ARDS patients. The toxicity of naked histones has been shown in various studies [23]. Zuo et al. detected elevated NET breakdown products in the serum of severe COVID-19 patients [24]. Histones also act as ligands for toll-like receptors on platelets and activate them [25]. The interaction of histones and platelet phospholipids activates the coagulation pathway [26].

NET has been associated with thrombi formation in the arterial-venous system with potentially fatal outcomes. Thus, when NETs circulate at high levels, a significant source of enzymatic activities can exaggerate the small-vessel occlusion. The activation of platelets can lead to enhanced NET formation by neutrophils in a vicious cycle during the patient's clinical deterioration leading to ARDS. Also, neutrophils can phagocytose antithrombin III and tissue factor pathway inhibitors [27]. Animal studies demonstrated that DNAase I dissolves the NET and reduces the thrombosis, improving the perfusion of the cardiac and renal vasculature. Therefore, NET can be a potential target in COVID-19 therapeutics (Figure *1*) [28,29].

2022 Agarwal et al. Cureus 14(9): e29760. DOI 10.7759/cureus.29760

2 of 9
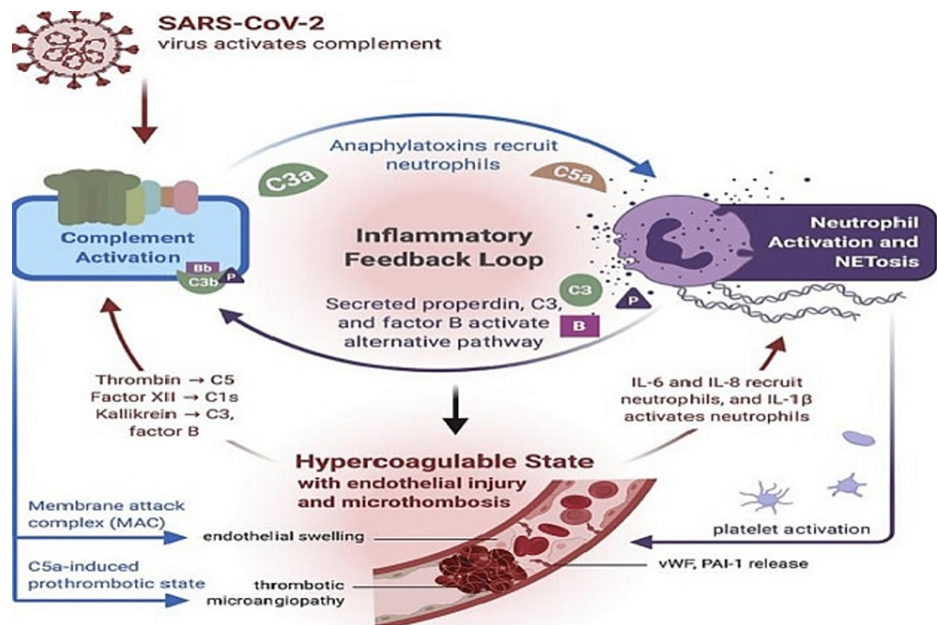
FIGURE 1: NET and COVID -19 disease

Image reproduced from: Java A, Apicelli AJ, Liszewski MK, Coler-Reilly A, Atkinson JP, Kim AH, Kulkarni HS. The complement system in COVID-19: friend and foe? JCI Insight. 2020 Aug 6;5(15):e140711. doi: 10.1172/jci.insight.140711.

Neutrophils and the cytokine storm

The severity of clinical conditions in COVID-19 cases has been repeatedly linked to cytokine storms. There is an increase in serum levels of interleukin (IL) 1β, IL-2, IL-6, IL-7, IL-8, IL-10, and IL-17 [30]. Also, serum levels of macrophage inflammatory protein 1α, tumor necrosis factor-α, interferon (IFN) γ, IFN-γ-inducible protein 10, and granulocyte colony-stimulating factor and monocyte chemoattractant protein 1 (MCP1) are elevated [31].

The neutrophilic NET formation, in turn, induces macrophages to increase the expression of IL-1β [31,32]. Thus, uncontrolled inflammatory reactions are achieved by continuous mutual induction of macrophages and neutrophils. Lachowicz-Scroggins et al. established an association between neutrophils and IL-1β in severe asthma patients, in whom COVID-19 can lead to progressive worsening of respiration decompensation and abnormal immune responses [33].

IL-8 can also induce neutrophils to release IL-6 receptor (R) α. Another potential target in COVID-19 therapeutics can be IL-6 induced by IL-1β (Figure 2) [34]. Calabrese and Rose-John demonstrated classic and trans-signaling mechanisms for IL-6. IL-6 interacts with IL-6Rα, and gp130 (a cytokine receptor) showed improved lung function in asthma patients with decreased plasma levels of IL-6Rα [35].
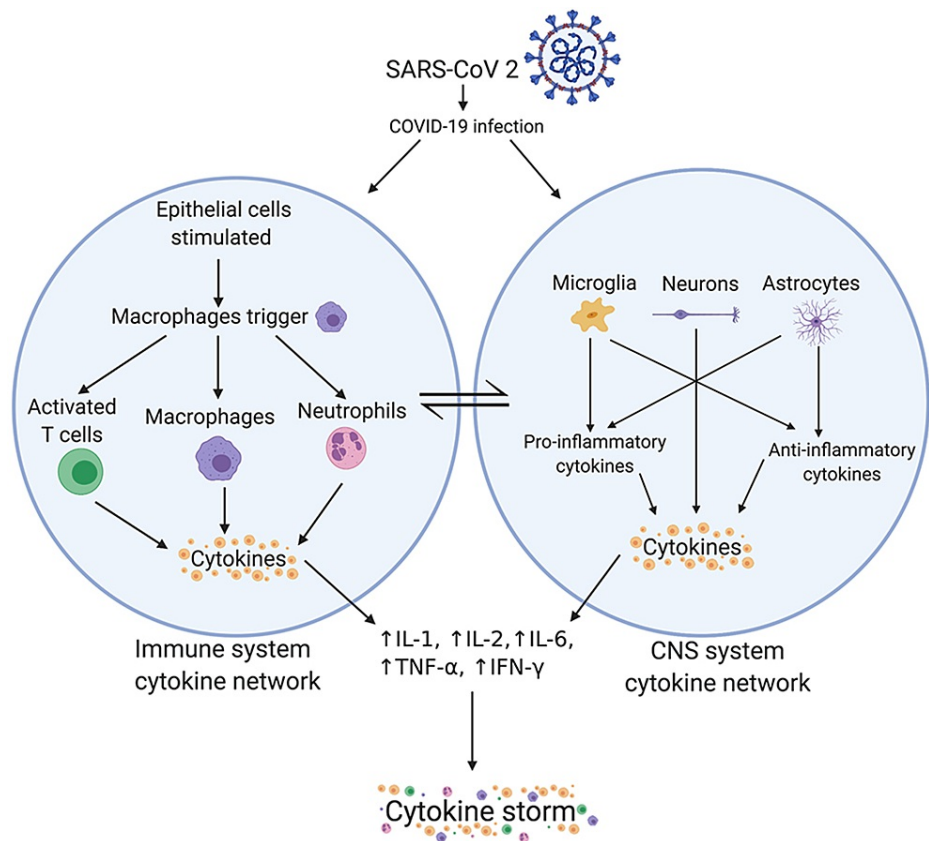
FIGURE 2: Netrophils and cytokine storm

Image reproduced from: Bhaskar S, Sinha A, Banach M, Mittoo S, Weissert R, Kass JS, Rajagopal S, Pai AR, Kutty S. Cytokine Storm in COVID-19-Immunopathological Mechanisms, Clinical Considerations, and Therapeutic Approaches: The REPROGRAM Consortium Position Paper. Front Immunol. 2020 Jul 10;11:1648. doi: 10.3389/fimmu.2020.01648.

Lymphopenia in SARS-CoV-2

The progression of the course of the disease leads to lymphopenia. The SARS-CoV-2 virus targets T lymphocytes, which is significant for a patient with critical conditions. CD8+ subsets of T cells show a marked decrease in the sera of patients admitted to the intensive care unit (ICU). Several studies have reported a close association between cytokine storm and reduction in levels of T cells [36,37]. Higher serum IL-6 levels have been associated with lower T lymphocytes and disease severity. Pathogenesis of lymphopenia in COVID-19 can be attributed to the inflammatory cytokine storm. A correlation has been observed between serum level of pro-inflammatory cytokines such as TNF-α and IL-6 and lymphopenia. Autopsy studies have noted severe lymphocyte destruction correlating with increased serum levels of IL-6 as well as Fas-FasL interactions. Studies reported impaired cytotoxic activity of natural killer (NK) cells and T cells significantly correlated with serum IL-6 levels [36]. Tocilizumab (an IL-6 receptor antagonist) showed promising results with significantly increased circulating lymphocytes [37]. Another mechanism can be T cell exhaustion in SARS-COV infections. Programmed cell death protein 1, T cell immunoglobulin, and mucin domain 3 are the markers of T cell exhaustion. These markers' increased cell surface expression was observed in CD+4 and CD+8 T cells in SARS-CoV-2 infections. Another suggested mechanism could be interference with T cell expansion. Decreased expression of CD107 and IFN-γ (T cell activation markers) was correlated to the severity of the disease, independently to several regulatory T cells. Downregulation of genes (MAP22K7, SOS1) associated with activation of T cell and function has been suggested in some studies. After recovery, gene expression returned to normal levels [38].

NLR as a prognostic tool

An immediate assessment of high-risk patients is needed after diagnosing SARS-CoV-2 infection. SARS-CoV-2 leads to an exaggerated immune response and significant damage to the host tissue by a cytokine storm. The rise in serum IL-1B, IFN-γ, IP10, and MCP1 has been reported. The decrease in CD+4 cells and CD+8 cells, and increased proinflammatory cytokines lead to even more significant lymphopenia. This correlates with severe clinical manifestation, immunosuppression, and NLR increase. Earlier studies have used NLR as a prognostic marker in diseases such as solid tumors, and lung, cardiovascular, and kidney diseases. Therefore, NLR can be used effectively as a simple, cost-effective systemic inflammatory marker in

laboratory investigations [39].

Ma et al. suggested that NLR can significantly differentiate severe disease from mild disease [40]. Wang et al. assessed the accuracy and sensitivity of NLR in detecting the severe cases at the time of hospital admission in a meta-analysis. They included 5,570 COVID-19 cases, including 1,607 severe cases. The sensitivity and specificity of NLR were 0.82 and 0.77, respectively. They concluded that NLR could accurately distinguish severe cases of COVID-19 from less severe cases [41]. NLR is a simple, cost-effective hematological tool. In another meta-analysis, NLR was reported to predict severe SARS-CoV-2 infections with accuracy [42]. Heterogeneity in the studies should be included to eliminate geographical and ethnic bias. Also, in most studies, the median patient age was 60 to 70 years. With age, the thymus function declines to approximately one-tenth of a younger adult, with progressive decline in the humoral and cellular immunity. Older patients are at a higher risk of severe infections and mortality than younger patients [43]. Singh et al. analyzed 201 laboratory-confirmed cases of SARS-CoV-2 and divided them into severe and non-severe groups based on clinical manifestation, shortness of breath, and oxygen saturation <93% with a ratio of arterial oxygen partial pressure to fractional inspired oxygen of ≤300 mmHg. NLR on day 1 and day 3 was 7.36 and 7.73, respectively, compared to NLR of 10.8 and 9.11 in the severe group. The overall sensitivity and specificity of NLR on day 1 were 60% and 55%, respectively. The disease progression and mortality rate was 4.4 times higher in the severe group than in the control group. NLR is a time-sensitive variable, and values are dynamic [44]. This supports another study that reported NLR was an independent inflammatory risk factor for mortality during hospitalization. NLR can accurately help identify severe cases early and allow timely intervention to reduce fatality. The results also reinforce the association between NLR and etiopathogenesis of SARS-CoV-2 [45]. Another study associated age and NLR to predict poor clinical outcomes and observed that patients (aged 50 or older) with NLR > 3.13 developed severe symptoms, while patients(aged 50 or older) with NLR < 3 and young patients showed better clinical outcomes than those admitted to the ICU. In a retrospective study of 74 laboratory-confirmed SARS-CoV-2 infections in Italy, improved clinical outcomes were predicted in the younger age group with NLR < 3. Severe clinical outcomes were observed in older patients with NLR > 4 [46]. Metacentric studies with large sample sizes should be conducted to eliminate potential biases.

Neutrophilia and lymphocytopenia are hallmarks of acute infection. Fox et al. noted neutrophil infiltration in the alveolar space, pulmonary capillaries, inflammation of mucosa with abundant neutrophils, and fibrin deposition in capillaries [47]. Barnes et al. proposed NETs as a potential mechanism for neutrophil-mediated damage [48]. Also, mechanical ventilation is required in severe COVID-19 with potential complications, as mechanical ventilation can itself cause alveolar damage [49]. Yildiz et al. noted NETs in mice models requiring mechanical ventilation [50].

Treatment strategies

Currently, the epidemic requires various combined drug therapeutics for severe COVID-19 cases and reduction of inflammation in every aspect of the inflammatory cycle and host response. Drugs targeting the neutrophilic egress and localization may reduce the clinical complications of alveolar damage and ARDS in COVID-19 cases. Earlier studies evaluated the role of Vitamin C infusion with limited benefits in the influx of granulocytes, activation, and NET formation. Clinical trials observed in vitro suppression of NET release by small peptides such as P140 [51]. Regulation of chaperone-mediated autophagy and macroautophagy by P140 has decreased inflammation in autoimmune disorders [52].

Important chemokine signaling of neutrophils' migration and the release of NET in the CXCL8/CXCR2 axis are under exploration [53]. Therefore, the CXCL8/CXCR2 axis antagonists have been evaluated in clinical trials of various respiratory distress conditions in influenza, eczema, and chronic obstructive pulmonary disease (COPD). Preclinical and clinical studies have tested the safety of AZD 5069 (a selective CXCR2 antagonist) [54] and SCH527123 [55] in COPD and asthma. The drug effectively blocked neutrophil egress and subsequent inflammatory activities in COPD patients. Other similar studies noted reduced neutrophilia with the use of danirixin (a CXCR2 inhibitor) [56,57] and navarixin (MK-7123/SCH 527123) [58] in phase 2 clinical trials in influenza and COPD cases. MS-986253 (a CXCL8 blocking antibody) is under investigation in COVID-19 patients [59].

Clinical trials have evaluated the potential role of sivelestat sodium (neutrophil elastase inhibitors) in patients with COPD and acute lung injury [60]. Sivelestat has been used with oseltamivir effectively in swine flu patients [61]. Elastase, a protease, degrades the proteins present in the alveolar basement membrane (e.g., elastin, collagen, and fibronectin) and aggravates alveolitis [62]. Additional lung tissue damage occurs by proteolytic priming of the viral glycoproteins, enabling membrane fusion in the host. Apart from the proteolytic effect, elastase has an important prothrombotic and proinflammatory role in the pulmonary vasculature [63].

Animal models of myeloproliferative tumors have evaluated the efficacy of peptidyl arginine deiminase IV (PAD4) inhibitors in reducing NET pathologies [64]. Some PAD4 inhibitors are Cl-amidine, YW-56, and GSK484. PAD4 converts arginine to citrulline in histones and promotes chromatin unwinding and NET release [65].

Recombinant DNase 1 can dissolve the NETs and provide some therapeutic benefits in combined therapeutic regimes [66]. The degradation products thus generated may also have proinflammatory activity [67], possibly mitigating inflammation spread and more significant tissue damage. Thus, further studies are required to evaluate the potential role of DNAse in severe SARS-CoV-2 infections.

Metoprolol is a β1 blocker shown to suppress NET-associated damage in the gall bladder. It also inhibits neutrophilic migration, reduces platelet aggregation and inflammation, and reduces infarct size in myocardial infarction cases [68]. Blocking the IL-1b/IL-1R interaction using anakinra (an IL-1R inhibitor) has shown promising results in initial clinical trials [69], reducing COVID-19 severity.

The C5a-C5aR1 interaction is another potential target [70]. The C5a molecule binds to the C5aR1 receptor and regulates neutrophilic activation and recruitment. The serum levels of these complement receptors are directly proportional to COVID-19 severity [71]. Vlaar et al. noted that the administration of IFX-1 (vilobelimab), a monoclonal antibody against C5a, and Solris (eculizumab), a C5 blocking antibody, shows adequate efficacy in the management of moderate and severe COVID-19 cases [72].

Corticosteroids have an anti-inflammatory effect and can improve lung tissue injury and ARDS. Systemic steroids administered to manage SARS-CoV-2 infections reduced neutrophil burst and recruitment at inflamed sites [73]. The immune regulation by corticosteroids is advisable but controversial since corticosteroid treatment could delay the clearance of SARS-CoV-2 in respiratory secretion or serum due to its immunosuppressive action [74]. Glucocorticoids have been effectively used in the treatment of SARS. In patients with COVID-19 and ARDS, methylprednisolone minimizes the risk of mortality. Li and Ma investigated the role of methylprednisolone in ARDS cases and found an increased risk of death [75].

Conclusions

NLR is an inflammatory marker and a proven diagnostic predictor of outcome and case severity in non-mild SARS-CoV-2 infections. With its wide availability, NLR assays can quickly assess a patient's condition and help the clinician alter treatments accordingly. Early stratification helps screen critically ill patients, as the higher the NLR value, the greater the need for support and care. Future studies should be conducted in different geographical settings to correlate outcomes with NLR and age, gender, hemodynamic variable, and comorbid conditions.

Additional Information

Disclosures

References

1. Fehr AR, Perlman S: Coronaviruses: an overview of their replication and pathogenesis . Methods Mol Biol. 2015, 1282:1-23. 10.1007/978-1-4939-2438-7_1
2. Wang N, Li SY, Yang XL, et al.: Serological evidence of bat SARS-related coronavirus infection in humans, China. Virol Sin. 2018, 33:104-7. 10.1007/s12250-018-0012-7
3. Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R: COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. J Adv Res. 2020, 24:91-8. 10.1016/j.jare.2020.03.005
4. Kumar D, Malviya R,Sharma PK: Corona virus: a review of COVID-19 . EJMO. 2020, 4:8-25. 10.14744/ejmo.2020.51418
5. Cao B, Wang Y, Wen D, et al.: A trial of lopinavir-ritonavir in adults Hospitalized with severe covid-19 . N Engl J Med. 2020, 382:1787-99. 10.1056/NEJMoa2001282
6. Wang X, Li X, Shang Y, et al.: Ratios of neutrophil-to-lymphocyte and platelet-to-lymphocyte predict all-cause mortality in inpatients with coronavirus disease 2019 (COVID-19): a retrospective cohort study in a single medical centre. Epidemiol Infect. 2020, 148:e211. 10.1017/S0950268820002071
7. Lin S, Mao W, Zou Q, Lu S, Zheng S: Associations between hematological parameters and disease severity in patients with SARS-CoV-2 infection. J Clin Lab Anal. 2021, 35:e23604. 10.1002/jcla.23604
8. Zhong X, Ma A, Zhang Z, Liu Y, Liang G: Neutrophil-to-lymphocyte ratio as a predictive marker for severe pediatric sepsis. Transl Pediatr. 2021, 10:657-65. 10.21037/tp-21-47
9. Bastug A, Bodur H, Erdogan S, et al.: Clinical and laboratory features of COVID-19: Predictors of severe prognosis. Int Immunopharmacol. 2020, 88:106950. 10.1016/j.intimp.2020.106950
10. Nacaroglu HT, İsgüder R, Bent S, et al.: Can neutrophil/lymphocyte ratio be a novel biomarker of inflammation in children with asthma?. Eur J Inflamm. 2016, 14:109-12. 10.1177/1721727X16660558
11. Shi G, Zhao JW, Ming L: [Clinical significance of peripheral blood neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in patients with asthma] [Article in Chinese]. Nan Fang Yi Ke Da Xue Xue Bao. 2017, 37:84-8. 10.3969/j.issn.1673-4254.2017.01.15
12. Xia X, Wen M, Zhan S, He J, Chen W: [An increased neutrophil/lymphocyte ratio is an early warning signal

of severe COVID-19]. Nan Fang Yi Ke Da Xue Xue Bao. 2020, 40:333-6. 10.12122/j.issn.1673-4254.2020.03.06

13. Ng LG, Ostuni R, Hidalgo A: Heterogeneity of neutrophils. Nat Rev Immunol. 2019, 19:255-65. 10.1038/s41577-019-0141-8

14. Kwok I, Becht E, Xia Y, et al.: Combinatorial single-cell analyses of granulocyte-monocyte progenitor heterogeneity reveals an early uni-potent neutrophil progenitor. Immunity. 2020, 53:303-318.e5. 10.1016/j.immuni.2020.06.005

15. Scapini P, Marini O, Tecchio C, Cassatella MA: Human neutrophils in the saga of cellular heterogeneity: insights and open questions. Immunol Rev. 2016, 273:48-60. 10.1111/imr.12448

16. Nicolás-Ávila JÁ, Adrover JM, Hidalgo A: Neutrophils in homeostasis, immunity, and cancer. Immunity. 2017, 46:15-28. 10.1016/j.immuni.2016.12.012

17. Wang R, Xiao H, Guo R, Li Y, Shen B: The role of C5a in acute lung injury induced by highly pathogenic viral infections. Emerg Microbes Infect. 2015, 4:e28. 10.1038/emi.2015.28

18. Sreeramkumar V, Adrover JM, Ballesteros I, et al.: Neutrophils scan for activated platelets to initiate inflammation. Science. 2014, 346:1234-8. 10.1126/science.1256478

19. Kuckleburg CJ, Tilkens SB, Santoso S, Newman PJ: Proteinase 3 contributes to transendothelial migration of NB1-positive neutrophils. J Immunol. 2012, 188:2419-26. 10.4049/jimmunol.1102540

20. Veras FP, Pontelli MC, Silva CM, et al.: SARS-CoV-2-triggered neutrophil extracellular traps mediate COVID-19 pathology. J Exp Med. 2020, 217:e20201129. 10.1084/jem.20201129

21. Chen N, Zhou M, Dong X, et al.: Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020, 395:507-13. 10.1016/S0140-6736(20)30211-7

22. Narasaraju T, Yang E, Samy RP, et al.: Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. Am J Pathol. 2011, 179:199-210. 10.1016/j.ajpath.2011.03.013

23. Xu J, Zhang X, Pelayo R, et al.: Extracellular histones are major mediators of death in sepsis. Nat Med. 2009, 15:1318-21. 10.1038/nm.2053

24. Zuo Y, Yalavarthi S, Shi H, et al.: Neutrophil extracellular traps in COVID-19. JCI Insight. 2020, 5:e138999. 10.1172/jci.insight.138999

25. Muller S, Radic M: Oxidation and mitochondrial origin of NET DNA in the pathogenesis of lupus. Nat Med. 2016, 22:126-7. 10.1038/nm.4044

26. Neeli I, Khan SN, Radic M: Histone deimination as a response to inflammatory stimuli in neutrophils. J Immunol. 2008, 180:1895-902. 10.4049/jimmunol.180.3.1895

27. Ashar HK, Mueller NC, Rudd JM, et al.: The role of extracellular histones in influenza virus pathogenesis. Am J Pathol. 2018, 188:135-48. 10.1016/j.ajpath.2017.09.014

28. Pedersen SF, Ho YC: SARS-CoV-2: a storm is raging. J Clin Invest. 2020, 130:2202-5. 10.1172/JCI137647

29. Schauer C, Janko C, Munoz LE, et al.: Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. Nat Med. 2014, 20:511-7. 10.1038/nm.3547

30. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ: COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020, 395:1033-4. 10.1016/S0140-6736(20)30628-0

31. Mikacenic C, Moore R, Dmyterko V, West TE, Altemeier WA, Liles WC, Lood C: Neutrophil extracellular traps (NETs) are increased in the alveolar spaces of patients with ventilator-associated pneumonia. Crit Care. 2018, 22:358. 10.1186/s13054-018-2290-8

32. Muñoz LE, Boeltz S, Bilyy R, et al.: Neutrophil extracellular traps initiate gallstone formation. Immunity. 2019, 51:443-450.e4. 10.1016/j.immuni.2019.07.002

33. Lachowicz-Scroggins ME, Dunican EM, Charbit AR, et al.: Extracellular DNA, neutrophil extracellular traps, and inflammasome activation in severe asthma. Am J Respir Crit Care Med. 2019, 199:1076-85. 10.1164/rccm.201810-1869OC

34. Rizk JG, Kalantar-Zadeh K, Mehra MR, Lavie CJ, Rizk Y, Forthal DN: Pharmaco-immunomodulatory therapy in COVID-19. Drugs. 2020, 80:1267-92. 10.1007/s40265-020-01367-z

35. Calabrese LH, Rose-John S: IL-6 biology: implications for clinical targeting in rheumatic disease. Nat Rev Rheumatol. 2014, 10:720-7. 10.1038/nrrheum.2014.127

36. Bordoni V, Sacchi A, Cimini E, et al.: An inflammatory profile correlates with decreased frequency of cytotoxic cells in coronavirus disease 2019. Clin Infect Dis. 2020, 71:2272-5. 10.1093/cid/ciaa577

37. Mazzoni A, Salvati L, Maggi L, et al.: Impaired immune cell cytotoxicity in severe COVID-19 is IL-6 dependent. J Clin Invest. 2020, 130:4694-703. 10.1172/JCI138554

38. Diao B, Wang C, Tan Y, et al.: Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). Front Immunol. 2020, 11:827. 10.3389/fimmu.2020.00827

39. Gong J, Dong H, Xia QS, et al.: Correlation analysis between disease severity and inflammation-related parameters in patients with COVID-19: a retrospective study. BMC Infect Dis. 2020, 20:963. 10.1186/s12879-020-05681-5

40. Ma A, Cheng J, Yang J, Dong M, Liao X, Kang Y: Neutrophil-to-lymphocyte ratio as a predictive biomarker for moderate-severe ARDS in severe COVID-19 patients. Crit Care. 2020, 24:288. 10.1186/s13054-020-03007-0

41. Wang Y, Zhao J, Yang L, Hu J, Yao Y: Value of the neutrophil-lymphocyte ratio in predicting COVID-19 severity: a meta-analysis. Dis Markers. 2021, 2021:2571912. 10.1155/2021/2571912

42. Lin L: Bias caused by sampling error in meta-analysis with small sample sizes. PLoS One. 2018, 13:e0204056. 10.1371/journal.pone.0204056

43. Bajaj V, Gadi N, Spihlman AP, Wu SC, Choi CH, Moulton VR: Aging, immunity, and COVID-19: how age influences the host immune response to coronavirus infections?. Front Physiol. 2020, 11:571416. 10.3389/fphys.2020.571416

44. Singh Y, Singh A, Rudravaram S, et al.: Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio as markers for predicting the severity in COVID-19 patients: a prospective observational study. Indian J Crit Care Med. 2021, 25:847-52. 10.5005/jp-journals-10071-23906

45. Wang C, Horby PW, Hayden FG, Gao GF: A novel coronavirus outbreak of global health concern. Lancet.

2020, 395:470-3. 10.1016/S0140-6736(20)30185-9

46. Liu J, Liu Y, Xiang P, et al.: Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. J Transl Med. 2020, 18:206. 10.1186/s12967-020-02374-0

47. Fox SE, Akmatbekov A, Harbert JL, Li G, Quincy Brown J, Vander Heide RS: Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. Lancet Respir Med. 2020, 8:681-6. 10.1016/S2213-2600(20)30243-5

48. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, et al.: Targeting potential drivers of COVID-19: Neutrophil extracellular traps. J Exp Med. 2020, 217:e20200652. 10.1084/jem.20200652

49. Akdogan RE, Mohammed T, Syeda A, Jiwa N, Ibrahim O, Mutneja R: Pneumothorax in mechanically ventilated patients with COVID-19 infection. Case Rep Crit Care. 2021, 2021:6657533. 10.1155/2021/6657533

50. Yildiz C, Palaniyar N, Otulakowski G, et al.: Mechanical ventilation induces neutrophil extracellular trap formation. Anesthesiology. 2015, 122:864-75. 10.1097/ALN.0000000000000605

51. Zimmer R, Scherbarth HR, Rillo OL, Gomez-Reino JJ, Muller S: Lupuzor/P140 peptide in patients with systemic lupus erythematosus: a randomised, double-blind, placebo-controlled phase IIb clinical trial. Ann Rheum Dis. 2013, 72:1830-5. 10.1136/annrheumdis-2012-202460

52. Macri C, Wang F, Tasset I, et al.: Modulation of deregulated chaperone-mediated autophagy by a phosphopeptide. Autophagy. 2015, 11:472-86. 10.1080/15548627.2015.1017179

53. Bendorius M, Neeli I, Wang F, et al.: The mitochondrion-lysosome axis in adaptive and innate immunity: effect of lupus regulator peptide P140 on mitochondria autophagy and NETosis. Front Immunol. 2018, 9:2158. 10.3389/fimmu.2018.02158

54. O'Byrne PM, Metev H, Puu M, et al.: Efficacy and safety of a CXCR2 antagonist, AZD5069, in patients with uncontrolled persistent asthma: a randomised, double-blind, placebo-controlled trial. Lancet Respir Med. 2016, 4:797-806. 10.1016/S2213-2600(16)30227-2

55. Holz O, Khalilieh S, Ludwig-Sengpiel A, et al.: SCH527123, a novel CXCR2 antagonist, inhibits ozone-induced neutrophilia in healthy subjects. Eur Respir J. 2010, 35:564-70. 10.1183/09031936.00048509

56. Madan A, Chen S, Yates P, et al.: Efficacy and safety of danirixin (gsk1325756) co-administered with standard-of-care antiviral (oseltamivir): a phase 2b, global, randomized study of adults hospitalized with influenza. Open Forum Infect Dis. 2019, 6:ofz163. 10.1093/ofid/ofz163

57. Pedersen F, Waschki B, Marwitz S, et al.: Neutrophil extracellular trap formation is regulated by CXCR2 in COPD neutrophils. Eur Respir J. 2018, 51:1700970. 10.1183/13993003.00970-2017

58. Rennard SI, Dale DC, Donohue JF, et al.: CXCR2 antagonist MK-7123. A phase 2 proof-of-concept trial for chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2015, 191:1001-11. 10.1164/rccm.201405-0992OC

59. Narasaraju T, Tang BM, Herrmann M, Muller S, Chow VT, Radic M: Neutrophilia and NETopathy as key pathologic drivers of progressive lung impairment in patients with COVID-19. Front Pharmacol. 2020, 11:870. 10.3389/fphar.2020.00870

60. Hayakawa M, Katabami K, Wada T, Sugano M, Hoshino H, Sawamura A, Gando S: Sivelestat (selective neutrophil elastase inhibitor) improves the mortality rate of sepsis associated with both acute respiratory distress syndrome and disseminated intravascular coagulation patients. Shock. 2010, 33:14-8. 10.1097/SHK.0b013e3181aa95c4

61. Yokoyama T, Tsushima K, Ushiki A, Kobayashi N, Urushihata K, Koizumi T, Kubo K: Acute lung injury with alveolar hemorrhage due to a novel swine-origin influenza A (H1N1) virus. Intern Med. 2010, 49:427-30. 10.2169/internalmedicine.49.3022

62. Gunawardena KA, Gullstrand H, Perrett J: Pharmacokinetics and safety of AZD9668, an oral neutrophil elastase inhibitor, in healthy volunteers and patients with COPD. Int J Clin Pharmacol Ther. 2013, 51:288-304. 10.5414/CP201674

63. Podolska MJ, Mahajan A, Hahn J, et al.: Treatment with DNases rescues hidden neutrophil elastase from aggregated NETs. J Leukoc Biol. 2019, 106:1359-66. 10.1002/JLB.3AB0918-370R

64. Li M, Lin C, Deng H, et al.: A novel peptidylarginine deiminase 4 (PAD4) inhibitor BMS-P5 blocks formation of neutrophil extracellular traps and delays Progression of multiple myeloma. Mol Cancer Ther. 2020, 19:1530-8. 10.1158/1535-7163.MCT-19-1020

65. Wolach O, Sellar RS, Martinod K, et al.: Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. Sci Transl Med. 2018, 10:8292. 10.1126/scitranslmed.aan8292

66. Cortjens B, de Jong R, Bonsing JG, van Woensel JB, Antonis AF, Bem RA: Local dornase alfa treatment reduces NETs-induced airway obstruction during severe RSV infection. Thorax. 2018, 73:578-80. 10.1136/thoraxjnl-2017-210289

67. Fisher J, Mohanty T, Karlsson CA, et al.: Proteome profiling of recombinant DNase therapy in reducing NETs and aiding recovery in COVID-19 patients. Mol Cell Proteomics. 2021, 20:100113. 10.1016/j.mcpro.2021.100113

68. García-Prieto J, Villena-Gutiérrez R, Gómez M, et al.: Neutrophil stunning by metoprolol reduces infarct size. Nat Commun. 2017, 8:14780. 10.1038/ncomms14780

69. Huet T, Beaussier H, Voisin O, et al.: Anakinra for severe forms of COVID-19: a cohort study . Lancet Rheumatol. 2020, 2:e393-400. 10.1016/S2665-9913(20)30164-8

70. Woodruff TM, Shukla AK: The complement C5a-C5aR1 GPCR axis in COVID-19 therapeutics . Trends Immunol. 2020, 41:965-7. 10.1016/j.it.2020.09.008

71. Carvelli J, Demaria O, Vély F, et al.: Association of COVID-19 inflammation with activation of the C5a-C5aR1 axis. Nature. 2020, 588:146-50. 10.1038/s41586-020-2600-6

72. Vlaar AP, de Bruin S, Busch M, et al.: Anti-C5a antibody IFX-1 (vilobelimab) treatment versus best supportive care for patients with severe COVID-19 (PANAMO): an exploratory, open-label, phase 2 randomised controlled trial. Lancet Rheumatol. 2020, 2:e764-73. 10.1016/S2665-9913(20)30341-6

73. Horby P, Lim WS, Emberson JR, et al.: Dexamethasone in hospitalized patients with Covid-19 . N Engl J Med. 2021, 384:693-704. 10.1056/NEJMoa2021436

74. Ronchetti S, Ricci E, Migliorati G, Gentili M, Riccardi C: How glucocorticoids affect the neutrophil life . Int J

2022 Agarwal et al. Cureus 14(9): e29760. DOI 10.7759/cureus.29760

8 of 9

Mol Sci. 2018, 19:4090. 10.3390/ijms19124090
75. Li X, Ma X: Acute respiratory failure in COVID-19: is it "typical" ARDS? . Crit Care. 2020, 24:198. 10.1186/s13054-020-02911-9

2022 Agarwal et al. Cureus 14(9): e29760. DOI 10.7759/cureus.29760

9 of 9