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The role of platelets and neutrophil extracellular traps (NETs) in sepsis: A comprehensive literature review

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Abstract

Sepsis is defined as "an organic dysfunction secondary to the dysregulated response of the patient to an infection." This concept only reveals the tip of the iceberg, the clinical expression of organic failures, without understanding their basis, which is currently explained by cellular and molecular phenomena. Neutrophils are crucial pillars of early innate immune responses, and their fundamental function is phagocytosis. Additionally, neutrophils can degranulate upon activation, releasing various antimicrobial enzymes and pro-inflammatory cytokines, and form neutrophil extracellular traps (NETs), whose purpose is to trap pathogens by releasing their "sticky" nuclear content; the presence of activated platelets amplifies this phenomenon. NETosis is a beneficial process; however, deregulated, it can be detrimental, inducing "immunothrombosis" and compromising the microcirculation, thereby increasing the clinical severity of sepsis. The purpose of this review is to clearly describe the pathophysiological role therapeutic target of NETs, their interaction with platelets in sepsis, and their potential as therapeutic targets, since it has been shown that a therapeutic approach aimed at curbing NETs would be beneficial.

Key word: sepsis, NETs, platelets.

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Introduction

Sepsis is currently defined (SEPSIS Consensus 3) as: "organ dysfunction secondary to a patient's dysregulated response to infection" (1). It is a major public health problem, with a mortality rate of 30-50%. It has been estimated that approximately 30 million people will suffer from sepsis each year, and 6 million will die from it (2).

In 2019, in the United States, a study on the incidence of sepsis in 6 hospital centers was conducted (3) where it was shown that of the 568 patients who were admitted, 52.8% of the cases were due to sepsis, reaching a mortality of 34%.

In Latin America, it is difficult to have precise bibliography since the difference at the time of choosing the diagnostic criteria is very wide; however, at the level of Colombia (4) it is reported an incidence of almost 80% of cases attended in the emergency was due to sepsis. In Mexico (5), sepsis has a mortality of 16%, while septic shock reaches 65% mortality.

In 2020, the Global Burden of Disease Study (6), a study that determines the incidence and mortality of sepsis from 1990 to 2017, indicated that in 2017 there were 48.9 million cases, and 11 million of these cases resulted in patient deaths, which represented 19.7% of all deaths worldwide that year, being more prevalent in sub-Saharan Africa, Oceania and Asia.

As we can analyze, sepsis mortality is still high, largely due to the heterogeneity of this disease, there is not a single form of presentation, so for 2019, Zhan et al (7) describes four phenotypes of sepsis according to its clinical presentation:

- Phenotype 1: individuals with low mortality rate and classic presentation.
- Phenotype 2: with respiratory dysfunction.
- Phenotype 3: multiple organ dysfunction, including coagulation compromise
- Phenotype 4: neurological dysfunction

Of these, as expected, phenotype 3 is the one associated with the highest mortality rate: 45%, even this group of patients curiously had a higher white blood cell count which leads to a higher concentration of neutrophils and even had a more significant alteration in the coagulation profile which makes us think that the state of immunothrombosis goes hand in hand with mortality, as we will see later the neutrophil-platelet interaction ends up being crucial for the development of this phenomenon.

The diagnostic approach to sepsis has gone from being purely clinical to being complimented and understood from a molecular point of view, in which PRR pattern recognition receptors (Toll-like and Nod-like receptors and others), pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) play a preponderant role and have enabled us to understand the intricate processes that induce tissue damage (8-11).

Within these molecular interactions, neutrophils, which are key cells in innate immunity, being professional phagocytes that destroy the phagocytosed pathogens by enzymatic mechanisms and dependent on reactive oxygen species (ROS) and other antimicrobial molecules (12), present a little-known but transcendental characteristic, which is the formation of extracellular traps (NETs), i.e., they commit suicide, throwing their DNA and enzymatic components into the capillaries, It has been demonstrated that this phenomenon depends on the type of NETS pathway that is formed, and even the effect will depend on the type of germ that generates the infection. It has been shown that if the germ is bacteria, the formation of NETS not only kills the bacteria but also limits their growth. Viral infections are characterized by excessive recruitment of neutrophils; therefore, the presence of NETS is more prevalent, which eliminates the virus and inhibits its replication. Platelets enhance this activity and, when regulated, become a relevant innate defense mechanism (13-15). However, when deregulated, it can produce a chaotic effect at the microcirculatory level and contribute to organ failure; thus an adverse effect on the formation of these traps has been demonstrated (15):

- Sepsis: Patients with NETS overexpression have been shown to have a higher mortality rate. The specific mechanism has not been determined, but it is believed that there is excessive apoptosis and necrosis, as well as decreased clearance of extruded products, and even the environment for a pro-inflammatory state that induces greater endothelial damage.
- Autoimmunity: NETS in diseases such as psoriasis, Lupus, and rheumatoid arthritis has been related to amplifying the inflammatory state.

This review aims to describe the individual behavior of NETS and their interaction with platelets in the pathophysiology of sepsis and, from this, to derive therapeutic targets and goals for treating this pathology.

Definitions

Platelets

Platelets are fragments of very large bone marrow cells, called megakaryocytes, which come from the myeloid lineage of the blood. The production of these cells is stimulated, for the most part, by thrombopoietin (TPO). As megakaryocytes develop into giant cells, they undergo a fragmentation process that results in the release of more than 1,000 platelets per megakaryocyte; once in circulation, platelets live for 8 to 10 days (16).

Normally, platelets are platelet-shaped (a French term that translates as plaque). When they are stimulated by a rupture in the blood vessel wall, they change shape: they become round and extend long filaments that serve to contact the wall of the ruptured blood vessel or with other platelets, thus forming a plug that will slow down bleeding, or stop it completely, and facilitate wound healing. This can be accomplished because they contain muscle-like proteins, which allow them to change shape, and other proteins on their surface, which allow them to adhere to breaks in the blood vessel wall and to adhere to each other. (17) They also contain alpha granules and dense granules that can secrete other proteins needed to create a firm plug to seal breaks in blood vessels. The alpha granules store factor V, factor VIII, von Willebrand factor, P-selectin, thrombospondin, fibrinogen, fibronectin, β-thromboglobulin, platelet-derived growth factor (PDGF), and platelet factor (16,17).

The dense granules contain calcium, adenosine diphosphate (ADP), and serotonin. In turn, the cytoplasm may contain other substances, such as serotonin, epinephrine, norepinephrine, nitric oxide, and cytokines. (16) However, hemostasis is not their only function.

Platelets are coupled with proteins/glycoproteins for various purposes, including hemostasis, thrombosis, sensing, natural anti-infectious defense (bacterial, viral, perhaps fungal), chemoattraction, cell communication, angiogenesis, healing, and tissue repair. (16) Thus, in innate immunity against bacterial products (or live bacteria as well), the TLR1, TLR2, TLR3, TLR4, TLR6, TLR7, and TLR9 pattern recognition receptors (PRRs) help platelets detect dangers of different natures and secrete different patterns of biological response modifiers (BRMs) accordingly; for example, LPS, recognized by TLR4, stimulates an intracellular signaling pathway that triggers the secretion of IL-1. (18)

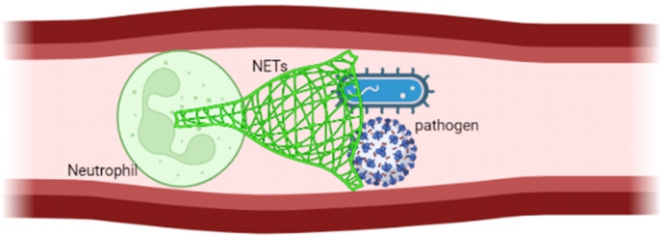
About 300 to 350 of the proteins currently known to

us are secreted by platelets. The proteins expressed in platelets have three origins: 1) inheritance from the megakaryocyte, 2) sponge effect, i.e., they are absorbed from neighboring fluids, especially plasma, and 3) by process of RNA retrotranscription and spliceosome, they can be produced de novo (13). In addition, platelets have also been reported to have novel roles in pathology: they make leukocytes prone to release neutrophil extracellular traps (platelet-induced NETosis) with functions in infection (e.g., sepsis) and in cancer, favoring thrombosis in either case (17).

NETosis

The term "NETosis" was first described in 2004 by German doctor Volker Brinkmann, who defined it as a new form of cell death (14,19). He observed that when stimulated by fungi or bacteria, neutrophils can produce networks composed of their own intracellular proteins, which they use to trap pathogenic components before dying (Figure 1). This mechanism has also been described in other cells of the immune system, such as basophils and eosinophils, and it has even been determined that macrophages also have this property since the release of these traps by these cells has been observed in the presence of staphylococcus aureus and hemolytic anemia(12,20).

Figure 1
Diagram of NETosis



In the presence of a pathogen (bacteria, virus, fungus), the neutrophil goes into action to exert its immunological effect, but apart from the classic action, it has been shown that these neutrophils also release networks that "engulf" these pathogens and help in their elimination.

Several stimuli determine the formation of NETs; phorbol-myristate-acetate (PMA), used in carcinogenic models, lipopolysaccharide, and interleukin-8 (IL-8) (14), were the first to be identified. However, at the present time, almost any infecting microorganism can trigger their genesis (21). Additionally, it has been demonstrated that certain neoplasms, such as pancreatic cancer, induce NETS formation through the glycosylation end product-dependent pathway (22), but not only has this mechanism been involved, it has been shown that NETS are closely related to the growth of neoplastic cells and their metastasis, It has been determined that NETS stimulate the box protein 1 of the high mobility group (HMGB1) which induces the epithelial to mesenchymal transition in tumor cells and, therefore, enhances their invasion capacity, likewise the NETS own proteinases can degrade the extracellular matrix which favors the extravasation of cancer cells and therefore their spread (23).

Types of NETs

Two basic types of NETs have been described, suicidal NETosis and vital NETosis. Currently, vital NETosis with an exclusive release of mitochondrial DNA and platelet-induced NETosis are being investigated (24).

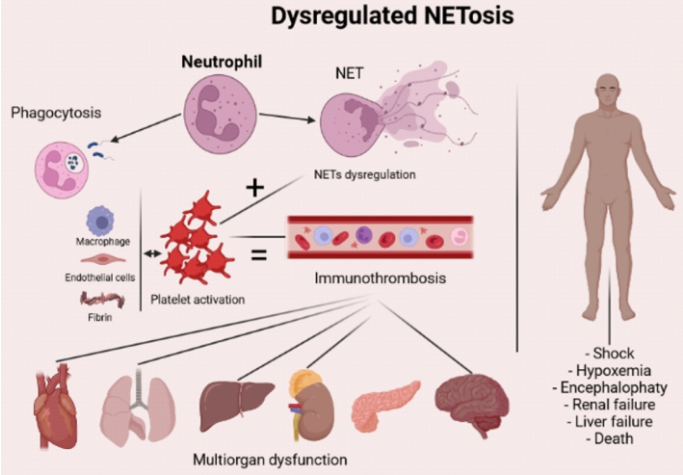
- **Suicidal NETosis**: requires the presence of NADPH-dependent ROS. This determines massive calcium entry into the neutrophil and its lysis by activating the enzyme peptidyl arginine deaminase 4 (PAD-4), which, together with myeloperoxidase and elastase transported to the nucleus, induces chromatin condensation and releases histones for subsequent release into the extracellular medium. (25,26)
- **Vital NETosis**: described in 2012, it is faster (minutes) than suicide (hours) and does not require the presence of ROS. It produces the release of NETs while maintaining the integrity of the nuclear and plasma membrane and, therefore, the phagocytic capacity of the neutrophil (21,22). Its usual triggers are TLRs and the receptor for complement fraction C3; this generates conformational changes in the nuclear membrane, which allows the release of vesicles loaded with nuclear DNA, which travels through the cytoplasm and is released into the extracellular space through the cell membrane (24,26-29).
- **Vital NETosis with mitochondrial DNA release**: described in 2009, it is activated by TLR-4 or complement receptor C5a and is probably facilitated by ROS.(30)
- **Platelet-triggered NETosis**: does not require ROS and is dependent on platelet glycoprotein IB and neutrophil beta2 integrin and involves von Willebrand factor. (31)

Platelets and NETs in sepsis

Activated platelets promote the recruitment of neutrophils to the site of injury (32) and the formation of neutrophil extracellular traps (NETs) that trap and help kill pathogens (33,34). Recently, extracellular histones were described as a cause of thrombocytopenia in critically ill patients (35). Fuchs et al. demonstrated that NETs, extracellular DNA fibers comprising histones and antimicrobial proteins from neutrophils, were formed at the endothelial level in infectious and noninfectious diseases (36). They reported that NETs provided a hitherto unknown stimulus for thrombus formation, generating platelet adhesion, activation, and aggregation, whereas NET formation in animal models caused rapid and profound thrombocytopenia. (37)

Platelet aggregation/adhesion to leukocytes and endothelial cells is a common mechanism for a type of thrombocytopenia called "immune thrombocytopenia" or "immunothrombosis" (Figure 2).

Figure 2
Neutrophil (NETosis)-platelet interaction that triggers immunothrombosis and systemic microcirculation involvement



In addition to their role in host defense, recent data suggest that NET formation contributes to the pathophysiology of many diseases, such as diabetes, autoimmune diseases, renal diseases, and heparin-induced thrombocytopenia (37-41). Sreeramkumar et al. reported that neutrophils recruited to the injured vessels of an animal model of inflammation interacted with activated platelets and endothelial cells (42). Their findings show a rapid and efficient regulatory mechanism in the early inflammation process. These observations underline the crucial role of platelet-leukocyte interactions in defense against infections during inflammation and sepsis. (42)

Toll-like receptors are known to promote the formation of NETs. They are a highly conserved family of pattern recognition receptors (PRRs) that bind pathogen-associated molecular patterns (PAMPs), molecules that are widely expressed by many infectious organisms. One of the most studied PAMPs is lipopolysaccharide (LPS), which is part of the gram-negative bacterial membrane and is a major ligand of TLR4. (43) Platelets express numerous members of the TLR family and, upon binding to their PAMPs, induce their activation, with subsequent release of IL-1 that induces platelet interaction with other cells. In addition, TLR2 activation induces the expression of adhesion molecules such as P-selectin and integrin IIb3, as well as the generation of reactive oxygen species (ROS) and the creation of cell aggregates composed of platelets and neutrophils. (43)

Sepsis creates a prothrombotic environment by promoting chaotic cellular events, including platelet, endothelial cell, and innate immune cell activation, NET formation, and fibrin production (40,45). These events generate the aforementioned "immunothrombosis", which initially represents an arm of innate immunity whose objective is to stop the dissemination and survival of pathogens; however, in a deregulated manner, it generates microvascular thrombosis and multi-organ dysfunction to a large extent. (46)

Detailing what has already been written in general lines, the cells of the innate immune system, fundamentally monocytes and neutrophils, are generators of immunothrombosis when they accumulate in small caliber vessels, lacerating the endothelium (glycocalyx and endothelial cells) and cause the release of tissue factor (which activates factor VII and induces the beginning of the coagulation cascade), destroying the natural anticoagulation system and finally building a procoagulant matrix which is constituted by extracellular nucleosomes; in this matrix, platelets adhere and activate which will form clots and trigger NETs, this pathway determines bacterial lysis at the microcirculation level. (47)

NETs are specifically procoagulant by activating coagulation factor XII (48) and eliminating key substances of the natural anticoagulant system, such as tissue factor pathway inhibitor (TFPI) and thrombomodulin (49).

In the bactericidal activity of immunothrombosis, fibrin is crucial since, per se, it has direct antimicrobial activity and limits the systemic dissemination of pathogens (50). The fibrin network traps and retains circulating microorganisms in the blood (bacteremias). There are several pathways of fibrin activation, one of which is through the extracellular nucleosomes of NETs. (51)

Factor XII is activated and converted to factor XIIa; at the same time, histones released in NETs can trigger platelet activation through TLR2 and TLR4. (48-50,52) Fuchs et al. were the first to demonstrate that NETs could induce the deposition of platelet adhesion molecules and the conversion of fibrinogen to fibrin. (36)

In COVID-19 and other viruses, neutrophils can produce NETs in a deregulated. Intense way, for example, the histones that are released in their formation are cytotoxic and induce greater damage to the endothelium. Even this accumulation of histones amplifies the damage at the pulmonary level and perpetuates thrombosis, this last phenomenon is interesting to analyze since it has been postulated that the scaffolds produced by NETS have an effect of platelet aggregation. Likewise, NETS releases serine proteases such as neutrophil elastase which enhances tissue factor and factor XII-dependent coagulation, leading to intravascular thrombus formation with thrombus formation and amplification of pro-inflammatory cytokine production, which aggravates the clinical manifestations of this entity by promoting the so-called "cytokine storm" (53,54).

Effects of dysregulated netosis in sepsis

In sepsis, neutrophils must reach the injured tissues; this is achieved by neutrophil-endothelial cell interaction; as these cells interact, IL-18 is released from the endothelium, which largely induces the formation of NETs, and these, in excess, induce endothelial damage (50). This has been demonstrated by blocking NETS NETs with NADPH oxidase or DNAase inhibitors and observing decreased endothelial damage. (56) When NET formation is deregulated, there is a harmful interaction with platelets, which aggregate

massively, activate thrombin and generate fibrin. The alteration of this axis, called NET-platelet-thrombin, makes the microcirculation chaotic, triggering diffuse microvascular thrombosis and disseminated intravascular coagulation, which accelerate the arrival of multi-organ dysfunction and cause greater clinical severity and mortality in these patients. (57-60) Blocking NETS NETs production by DNAase improved tissue perfusion by permeabilizing capillaries and decreased multi-organ damage. (57)

A novel finding is that extravascular NETosis has been detected, that is, when neutrophils migrate to tissues, for example, in the alveolar epithelium of septic humans and animals (dogs and mice), by bronchoalveolar lavage NETs and histones were detected; when these histones were administered in healthy alveolar tissue, the inflammatory lesion was induced, which supports the damaging power of unregulated NETosis (61-65). Added to the above, neutrophil enzymes that are expelled during NETosis, such as elastase and serine protease, degrade the alveolar epithelium. The first is by increasing the permeability of the alveolar cells, which leads to a water imbalance, with fluid accumulation and increased gravity (65), and the second is by destroying the surfactant, which increases the surface tension forces and leads to alveolar collapse (66).

Translational approach and therapeutic strategies targeting NETs

Currently, treatment of sepsis consists of supportive care and antibiotics, and none has focused on the host response, which is the major cause of death in sepsis. Although neutrophils show dysregulated function during sepsis, they also have a longer life expectancy, making them a potential therapeutic target (67).

Therapeutic strategies targeting NETs mainly target the DNA component: DNAase is the most frequent treatment modality. DNAase treatment reduced NETs, improving lung injury and survival in a murine model of pneumonia (68); although early DNAase administration worsened outcomes in the CLP (cecum ligation and puncture) model, combined antibiotic and DNAase treatment resulted in improved survival, reduced bacteremia, and less organ dysfunction (69). This indicates that combination therapies that include conventional treatment and drugs targeting NETs can potentially optimize the efficacy and outcome of treatment in septic patients.

Genetic or pharmacological inhibition of other NET-forming factors, such as PAD4, has been shown to improve survival (70). However, since NET formation is also important for pathogen control, maintaining an adequate amount of NETs to meet the demand for bacterial control and also to prevent tissue injury becomes an emerging area for the discovery of targeted therapies. Cl-Amidine, a PAD4 inhibitor, had no effect on the level of neutrophil-DNA complexes or the degree of lung inflammation in a murine model of pneumonia, but research showed that it prevented H3 citrullination, NET formation, and improved survival in a murine model of CLP-induced polymicrobial sepsis (68). Similarly, mice with a total deficiency of PAD4 (PAD4 -/-) demonstrated decreased NETs and lung injury in the pneumonia model. However, these benefits were accompanied by increased bacterial load and systemic inflammation. This is why Lefrancais et al. developed a mouse with a partial deficiency of PAD4 (PAD4 +/-) that demonstrated an improved survival curve. (68)

Blockade of NETs components, such as histones, benefits survival in the animal model (71-73). In contrast, large-scale randomized clinical trials evaluating efficacy in human patients with sepsis failed to demonstrate any clinical benefit (73). Other inhibitors targeting extracellular histones have recently been tested, with promising results identified (74). Chloroquine has also been effective as an early inhibitor of NETs, decreasing NETosis and associated hypercoagulability and improving survival in murine models of pancreatic adenocarcinoma and acute pancreatitis (75,76).

Table 1

Summary of therapeutic strategies targeting NETs (77)

Objective	Strategy	Results	References
NETs	DNAasa I	Effective when combined with antibiotics to improve outcome	Czaikoski y col. (2016)
	Cl-Amidina	Prevents NET formation and improves survival in a mouse CLP model	Biron y col. (2017)
	Histona 3 anti-citrulinada	Reduced TNE and improved survival in a mouse CLP model	Li y col. (2014)
	Anti – TREM-1	Prevents NETosis and associated endothelial dysfunction in a mouse LPS model	Murao y col. (2020), Boufenzer et al. (2021)
Histones	Antihistona	Improved outcome in LPS, TNF, and CLP mouse models	Meara y col. (2020)
	Activated protein C	Failed in the clinic	Marti-Carvajal y col. (2012)
Platelets	Acetylsalicylic acid	Decreases intravascular NETosis and tissue injury	Caudrillier et al. (2012)
	Anti-P2Y12	Prevents NETosis and improves survival in CLP model mice	Liverani y col. (2016), Mansour et al. (2020)
	Antiplatelet factor 4	Stabilizes NETs and prevents the release of antibacterial compounds in a mouse model	Gollomp y col. (2020)

Because platelet-neutrophil interaction is crucial for NETosis, antiplatelet therapy may also be an interesting field to investigate. Platelet activation requires eicosanoids such as thromboxane A2; blockade of thromboxane A2 generation with the use of acetylsalicylic acid or aspirin has been shown to decrease intravascular NETosis and tissue injury (77). Other inhibitors that target platelet-neutrophil interaction, such as the platelet ADP receptor, P2Y12, also attenuated NETosis (78,79). Furthermore, in several observational studies, the administration of antiplatelet therapy has been shown to be associated with improved outcomes in patients with sepsis (80). Recently, a novel treatment has been discovered that stabilizes NETs to improve their ability to capture bacteria and prevent the release of antibacterial compounds that cause tissue damage (81). The researchers developed an antibody that binds to complexes of NETs and platelet factor 4 (PF4), a protein released by activated platelets, which makes NETs resist degradation and enhances their ability to capture bacteria. When administered in combination with antibiotics, this treatment significantly reduces the severity of the disease, decreases the levels of bacteria circulating in the blood, and improves survival in the animal sepsis model (Table 1). (82)

Conclusion

Netosis generated by neutrophils is part of the defense mechanisms of innate immunity, helps control the microbial load, and enhances the initial defense against pathogens; however, its deregulation can lead to severe microvascular alterations that can ultimately induce multiple organ failure and even death.

Sepsis remains a disease of high prevalence and high mortality rates; no treatment fits all patients, and this is due to its heterogeneity; however, this understanding that there is a subtype that is related to more multi-organ damage makes us think that molecular phenomena and in particular the excessive formation of NETS are key to its lethality, so developing targeted treatments against these NETS would help us mitigate the mortality of this disease.

Knowledge of this phenomenon has generated therapeutic strategies that primarily target NETs and histones or platelets as coadjuvant fragments. The results are not yet categorical, but they open a new possibility of pharmacological intervention in sepsis and other human pathologies.

Authors' contributions

JV, FR, SD, AR, and NK contributed to the literature review, selection of articles, and drafting of the manuscript as well as to the preparation of tables and drawings; FR and SD were in charge of consolidating and arranging the bibliographic citations according to the indicated standards, JV, AR, NK made the final revision and all authors reviewed the results and agreed to submit them for publication, accepting to be responsible for each aspect written in the present article.

Ethics statement

The authors declare that the published work reflects an investigation and analysis carried out truthfully and completely.

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Conflict of interest

The authors declare no conflict of interest.

Availability of data

NA.

References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. (2016) 315:801–10. doi: 10.1001/jama.2016.0287 CrossRef Full Text | Google Scholar
2. Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, et al. Assessment of global incidence and mortality of hospital-treated sepsis. current estimates and limitations. Am J Respir Crit Care Med. (2016) 193:259–72. doi: 10.1164/rccm.201504-0781OC
3. Rhee C, Jones TM, Hamad Y, et al. Prevalence, Underlying Causes, and Preventability of Sepsis-Associated Mortality in US Acute Care Hospitals. JAMA Netw Open. 2019;2(2):e187571. doi:10.1001/jamanetworkopen.2018.7571
4. Jaimes F, Garcés J, Cuervo J, Ramírez F, Ramírez J, Vargas A, et al. The systemic inflammatory response syndrome (SIRS) to identify infected patients in the emergency room. Intensive Care Med. 2003;29:136871.
5. Gorordo-Delsol, Luis A., Merinos-Sánchez, Graciela, Estrada-Escobar, Ricardo A., Medveczky-Ordoñez, Nikolett I., Amezcua-Gutiérrez, Marcos A., Morales-Segura, Ma. Angélica, & Uribe-Moya, Silvia E.. (2020). Sepsis y choque séptico en los servicios de urgencias de México: estudio multicéntrico de prevalencia puntual. Gaceta médica de México, 156(6), 495-501. Epub 27 de mayo de 2021.https://doi.org/10.24875/gmm.19005468
6. Rudd, K. E., Johnson, S. C., Agesa, K. M., Shackelford, K. A., Tsoi, D., Kievlan, D. R., Colombara, D. V., Ikuta, K. S., Kissoon, N., Finfer, S., Fleischmann-Struzek, C., Machado, F. R., Reinhart, K. K., Rowan, K., Seymour, C. W., Watson, R. S., West, T. E., Marinho, F., Hay, S. I., … Naghavi, M. (2020). Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. Lancet, 395(10219), 200–211. https://doi.org/10.1016/S0140-6736(19)32989-7
7. Zhang, Z., Zhang, G., Goyal, H. et al. Identification of subclasses of sepsis that showed different clinical outcomes and responses to amount of fluid resuscitation: a latent profile analysis. Crit Care 22, 347 (2018). https://doi.org/10.1186/s13054-018-2279-3

8. Aziz M, Jacob A, Yang WL, Matsuda A, Wang P. Current trends in inflammatory and immunomodulatory mediators in sepsis. J Leukoc Biol. (2013) 93:329–42. doi: 10.1189/jlb.0912437
9. Cabrera-Perez J, Badovinac VP, Griffith TS. Enteric immunity, the gut microbiome, and sepsis, Rethinking the germ theory of disease. Exp Biol Med. (2017) 242:127–39. doi: 10.1177/1535370216669610
10. Gentile LF, Moldawer LL. DAMPS, PAMPS and the origins of SIRS in bacterial sepsis. Shock. (2013) 39:113– 4. doi: 10.1097/SHK.0b013e318277109c
11. Takeuchi O, Akira, S. Pattern recognition receptors and inflammation. Cell. (2010) 140:805–20. doi: 10.1016/j.cell.2010.01.022
12. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. (2013) 13:159–75. doi: 10.1038/nri3399
13. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol. (2018) 18:134–47. doi: 10.1038/nri.2017.105
14. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. (2004) 303:1532–5. doi: 10.1126/science.1092385 PubMed Abstract | CrossRef Full Text | Google Scholar
15. Mutua, V., & Gershwin, L. J. (2021). A Review of Neutrophil Extracellular Traps (NETs) in Disease: Potential Anti-NETs Therapeutics. Clinical reviews in allergy & immunology, 61(2), 194–211. https://doi.org/10.1007/s12016-020-08804-7
16. Ghoshal K, Bhattacharyya M. Overview of Platelet Physiology: Its Hemostatic and Nonhemostatic Role in Disease Pathogenesis. The Scientific World Journal. (2014) 2014:16. doi: 10.1155/2014/781857 Publisher Site
17. Fong KP, Barry C, Tran AN, Traxler EA, Wannemacher KM, Tang H, et al. Deciphering the human platelet sheddome. Blood. (2011) 117(1): e15–e26. doi: 10.1182/blood-2010-05-283838 Publisher Site
18. Garraud O, Cognasse F. Are Platelets Cells? And if Yes, are They Immune Cells? Frontiers in Immunology. (2015) 6: 70. doi: 10.3389/fimmu.2015.00070 Publisher Site
19. Sánchez-Zúñiga MJ. Netosis. Revista Mexicana de Anestesiología. (2019) 42: S47-S49. Publisher Site
20. Vorobjeva NV, Chernyak BV. NETosis: Molecular Mechanisms, Role in Physiology and Pathology. Biochemistry (Mosc). (2020) 85(10):1178-1190. doi: 10.1134/S0006297920100065 PMC free article
21. Brinkmann V. Neutrophil extracellular traps in the second decade. J Innate Immun. (2018) 10:414–21. doi: 10.1159/000489829 PubMed Abstract | CrossRef Full Text | Google Scholar
22. Boone BA, Orlichenko L, Schapiro NE, Loughran P, Gianfrate GC, Ellis JT, et al. The receptor for advanced glycation end products (RAGE) enhances autophagy and neutrophil extracellular traps in pancreatic cancer. Cancer Gene Ther. (2015) 22:326–34. doi: 10.1038/cgt.2015.21 PubMed Abstract | CrossRef Full Text | Google Scholar
23. Demkow U. Neutrophil Extracellular Traps (NETs) in Cancer Invasion, Evasion and Metastasis. Cancers (Basel). 2021;13(17):4495. Published 2021 Sep 6. doi: 10.3390/cancers13174495
24. Godínez VAR, Carrillo ER, Cabello AR. Trampas extracelulares de neutrófilos. Medicina Crítica. (2020) 34(2):156-159. doi: 10.35366/93970 Publisher Site
25. Yipp BG, Kubes P. NETosis: how vital is it? Blood. (2013) 122:2784–94. doi: 10.1182/blood-2013-04-457671 PubMed Abstract | CrossRef Full Text | Google Scholar
26. 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
27. Delgado-Rizo V, Martinez-Guzman MA, Iniguez-Gutierrez L, Garcia-Orozco A, Alvarado-Navarro A, Fafutis-Morris M. Neutrophil extracelular traps and its implications in inflammation: an overview. Front Immunol. (2017) 8:81. doi: 10.3389/fimmu.2017.00081
28. Yipp BG, Petri B, Salina D, Jenne CN, Scott BN, Zbytnuik LD, et al. Infectioninduced NETosis is a dynamic process involving neutrophil multitasking in vivo. Nat Med. (2012) 18:1386–93. doi: 10.1038/nm.2847
29. Manda, Pruchniak MP, Arazna M, Demkow UA. Neutrophil extracelular traps in physiology and pathology. Cent Eur J Immunol. (2014) 39:116–21. doi: 10.5114/ceji.2014.42136
30. de Buhr N, von Köckritz-Blickwede M. How neutrophil extracellular traps become visible. J Immunol Res. (2016) 2016:4604713. doi: 10.1155/2016/4604713
31. Yousefi S, Mihalache C, Kozlowski E, Schmid I, Simon HU. Viable neutrophils release mitochondrial DNA to form neutrophil extracelular traps. Cell Death Differ. (2009) 16:1438–44. doi: 10.1038/cdd.2009.96
32. Carestia A, Kaufman T, Rivadeneyra L, Landoni VI, Pozner RG, Negrotto S, et al. Mediators and molecular pathways involved in the regulation of neutrophil extracellular trap formation mediated by activated platelets. J Leukoc Biol. (2016) 99:153–62. doi: 10.1189/jlb.3A0415-161R
33. Grommes, J.; Alard, J.-E.; Drechsler, M.; Wantha, S.; Mörgelin, M.; Kuebler, W.M.; Jacobs, M.; von Hundelshausen, P.; Markart, P.;Wygrecka, M.; et al. Disruption of platelet-derived chemokine heteromers prevents neutrophil extravasation in acute lung injury. Am. J. Respir. Crit. Care Med. 2012, 185, 628–636. [CrossRef] [PubMed]
34. Ma, AC; Kubes, P. Platelets, neutrophils, and neutrophil extracellular traps (NETs) in sepsis. J. Thromb. Haemost. 2008, 6, 415–420. [CrossRef] [PubMed]
35. Alhamdi, Y.; Toh, C.-H. The role of extracellular histones in haematological disorders. Br. J. Haematol. 2016,173, 805–811. [CrossRef]
36. Fuchs, T.A.; Brill, A.; Duerschmied, D.; Schatzberg, D.; Monestier, M.; Myers, D.D.; Wrobleski, S.K.; Wakefield, T.W.; Hartwig, J.H.; Wagner, D.D. Extracellular DNA traps promote thrombosis. Proc. Natl. Acad. Sci. USA 2010, 107, 15880–15885. [CrossRef]
37. Zhu, L.; Liu, L.; Zhang, Y.; Pu, L.; Liu, J.; Li, X.; Chen, Z.; Hao, Y.; Wang, B.; Han, J.; et al. High Level of Neutrophil Extracellular Traps Correlates With Poor Prognosis of Severe Influenza A Infection. The Journal of Infectious Diseases 2018, 217, 428–437. [CrossRef] [PubMed]
38. McDonald, B.; Davis, R.P.; Kim, S.-J.; Tse, M.; Esmon, CT; Kolaczkowska, E.; Jenne, C.N. Platelets and neutrophil

extracellular traps collaborate to promote intravascular coagulation during sepsis in mice. Blood 2017, 129, 1357–1367. [CrossRef] [PubMed]
39. Fadini, G.P.; Menegazzo, L.; Rigato, M.; Scattolini, V.; Poncina, N.; Bruttocao, A.; Ciciliot, S.; Mammano, F.; Ciubotaru, C.D.; Brocco, E.; et al. NETosis Delays DiabeticWound Healing in Mice and Humans. Diabetes 2016, 65, 1061–1071. [CrossRef] Int. J. Mol. Sci. 2019, 20, 3494 11 of 13
40. Gupta, S.; Kaplan, M.J. The role of neutrophils and NETosis in autoimmune and renal diseases. Nat. Rev. Nephrol. 2016, 12, 402–413. [CrossRef] [PubMed]
41. Perdomo, J.; Leung, H.H.L.; Ahmadi, Z.; Yan, F.; Chong, J.J.H.; Passam, F.H.; Chong, B.H. Neutrophil activation and NETosis are the major drivers of thrombosis in heparin-induced thrombocytopenia. Nat. Commun. 2019, 10, 1322. [CrossRef] [PubMed]
42. Sreeramkumar, V.; Adrover, J.M.; Ballesteros, I.; Cuartero, M.I.; Rossaint, J.; Bilbao, I.; Nácher, M.; Pitaval, C.; Radovanovic, I.; Fukui, Y.; et al. Neutrophils scan for activated platelets to initiate inflammation. Science 2014, 346, 1234–1238. [CrossRef] [PubMed]
43. Blair, P.; Rex, S.; Vitseva, O.; Beaulieu, L.; Tanriverdi, K.; Chakrabarti, S.; Hayashi, C.; Genco, C.A.; Iafrati, M.; Freedman, J.E. Stimulation of Toll-like receptor 2 in human platelets induces a thromboinflammatory response through activation of phosphoinositide 3-kinase. Circ. Res. 2009, 104, 346–354. [CrossRef] [PubMed]
44. McDonald, B.; Urrutia, R.; Yipp, B.G.; Jenne, C.N.; Kubes, P. Intravascular Neutrophil Extracellular Traps Capture Bacteria from the Bloodstream during Sepsis. Cell Host Microbe 2012, 12, 324–333. [CrossRef] [PubMed]
45. Semeraro, F.; Ammollo, C.T.; Morrissey, J.H.; Dale, G.L.; Friese, P.; Esmon, NL; Esmon, CT Extracellular histones promote thrombin generation through platelet-dependent mechanisms: Involvement of platelet TLR2 and TLR4. Blood 2011, 118, 1952–1961. [CrossRef] [PubMed]
46. Engelmann, B.; Massberg, S. Thrombosis as an intravascular e_ector of innate immunity. Nat. Rev. Immunol.2012, 13, 34–45. [CrossRef] [PubMed]
47. Camicia, G.; Pozner, R.; de Larrañaga, G. Neutrophil extracellular traps in sepsis. Shock 2014, 42, 286–294. [CrossRef]
48. von Brühl, M.L.; Stark, K.; Steinhart, A.; Chandraratne, S.; Konrad, I.; Lorenz, M.; Khandoga, A.; Tirniceriu, A.; Coletti, R.; Köllnberger, M.; et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. J. Exp. Med. 2012, 209, 819–835. [CrossRef]
49. Massberg, S.; Grahl, L.; von Bruehl, M.L.; Manukyan, D.; Pfeiler, S.; Goosmann, C.; Brinkmann, V.; Lorenz, M.; Bidzhekov, K.; Khandagale, A.B.; et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. Nat. Med. 2010, 16, 887–896. [CrossRef]
50. Zinsser, H.; Pryde, AW. Experimental study of physical factors, including fibrin formation, influencing the spread of fluids and small particles within and from the peritoneal cavity of the dog. Ann. Surg. 1952, 136, 818–827. [CrossRef]
51. Engelmann, B. Extracellular DNA and histones as thrombus stabilizer. Thromb. Haemost. 2015, 113, 1164. [PubMed]
52. Xu, J.; Zhang, X.; Monestier, M.; Esmon, NL; Esmon, CT Extracellular histones are mediators of death through TLR2 and TLR4 in mouse fatal liver injury. J. Immunol. 2011, 187, 2626–2631. [CrossRef] [PubMed]
53. Fajgenbaum D, June, C. Cytokine Storm. N Engl J Med 2020; 383:2255-2273 DOI: 10.1056/NEJMra2026131
54. Ventura-Santana, E., Ninan, J. R., Snyder, C. M., & Okeke, E. B. (2022). Neutrophil extracellular traps, sepsis and COVID-19 - A tripod stand. Frontiers in Immunology, 13, 902206. https://doi.org/10.3389/fimmu.2022.902206
55. Lerman YV, Kim M. Neutrophil Migration under normal and sepsis conditions. Cardiovasc Hematol Disord Drug Targets. (2015) 15:19–28. doi: 10.2174/1871529X15666150108113236
56. Gupta K, Joshi MB, Philippova M, Erne P, Hasler P, Hahn S, Resink TJ. Activated endothelial cells induce neutrophil extracellular traps and are susceptible to NETosis-mediated cell death. FEBS Lett. (2010) 584:3193– 7. doi: 10.1016/j.febslet.2010.06.006
57. Kimball S, Obi AT, Diaz JA, Henke PK. The emerging role of NETs in venous thrombosis and immunothrombosis. Front Immunol. (2016) 7:236. doi: 10.3389/fimmu.2016.00236
58. Martinod K, Wagner DD. Thrombosis: tangled up in NETs. Blood. (2014) 123:2768–76. doi: 10.1182/blood-2013-10-463646
59. Delabranche X, Stiel L, Severac F, Galoisy AC, Mauvieux L, Zobairi F, et al. Evidence of netosis in septic shock-induced disseminated intravascular coagulation. Shock. (2017) 47:313–17. doi: 10.1097/SHK.0000000000000719
60. McDonald A, Davis RP, Kim SJ, Tse M, Esmon CT, Kolaczkowska E, et al. Platelets and neutrophil extracellular traps collaborate to promote intravascular coagulation during sepsis in mice. Blood. (2017) 129:1357– 67. doi: 10.1182/blood-2016-09-741298
61. Yang S, Qi H, Kan K, Chen J, Xie H, Guo X, et al. Neutrophil extracelular traps promote hypercoagulability in patients with sepsis. Shock. (2017) 47:132–9. doi: 10.1097/SHK.0000000000000741
62. Mikacenic A, Moore R, Dmyterko V, West TE, Altemeier WA, Liles WC, et al. Neutrophil extracellular traps (NETs) are increased in the alveolar spaces of patients with ventilator-associated pneumonia. Crit Care. (2018) 22:358. doi: 10.1186/s13054-018-2290-8
63. Li RH L, Johnson LR, Kohen C, Tablin F. A novel approach to identifying and quantifying neutrophil extracellular trap formation in septic dogs using immunofluorescence microscopy. BMC Vet Res. (2018) 14:210. doi: 10.1186/s12917-018-1523-z
64. Bosmann M, Grailer JJ, Ruemmler R, Russkamp NF, Zetoune FS, Sarma JV, et al. Extracellular histones are essential effectors of C5aR- and C5L2- mediated tissue damage and inflammation in acute lung injury. FASEB J. (2013) 27:5010–21. doi: 10.1096/fj.13-236380
65. Peterson MW, Walter ME, Nygaard SD. Effect of neutrophil mediators on epithelial permeability. Am J Respir Cell Mol Biol. (1995) 13:719–27. doi: 10.1165/ajrcmb.13.6.7576710
66. Li RHL, Tablin F. A comparative review of neutrophil extracellular traps in sepsis. Front Vet Sci. (2018) 5:291. doi: 10.3389/fvets.2018.00291
67. Shen XF, Cao K, Jiang JP, Guan WX, Du JF. Neutrophil

dysregulation during sepsis: an overview and update. Journal of Cellular and Molecular Medicine. (2017) 21(9):1687-1697. doi:10.1111/jcmm.13112 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
68. Lefrancais E, Mallavia B, Zhuo H, Calfee CS, Looney MR. Maladaptive role of neutrophil extracellular traps in pathogen-induced lung injury. JCI Insight. (2018) 3:98178. doi: 10.1172/jci.insight.98178 [PubMed Abstract] [CrossRef Full Text] [Google Scholar]
69. Czaikoski P. G., Mota J. M., Nascimento D. C., Sonego F., Castanheira F. V., Melo P. H., et al. Neutrophil extracellular traps induce organ damage during experimental and clinical sepsis. PLoS One. (2016). 11 (2): e0148142. doi: 10.1371/journal.pone.0148142 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
70. Martinod K., Fuchs T. A., Zitomersky N. L., Wong S. L., Demers M., Gallant M., et al. PAD4-deficiency does not affect bacteremia in polymicrobial sepsis and ameliorates endotoxemic shock. Blood. (2015). 125 (12): 1948–1956. doi: 10.1182/blood-2014-07-587709 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
71. Biron BM, Chung CS, O'Brien XM, Chen Y, Reichner JS, Ayala A. Cl-amidine prevents histone 3 citrullination, net formation, and improves survival in a murine sepsis model. Journal of Innate Immunity. (2017) 9:22–32. doi: 10.1159/000448808 [CrossRef Full Text] [Google Scholar]
72. Semeraro F, Ammollo CT., Morrissey JH, Dale GL, Friese P, Esmon NL, et al. Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. Blood. (2011). 118(7):1952–1961. doi: 10.1182/blood-2011-03-343061 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
73. Li Y, Liu Z, Liu B, Zhao T, Chong W, Wang Y, et al. Citrullinated histone H3: a novel target for the treatment of sepsis. Surgery. (2014). doi: 156(2):229–234. 10.1016/j.surg.2014.04.009 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
74. Martí-Carvajal AJ, Solà I, Gluud C, Lathyris D, Cardona AF. Human recombinant protein C for severe sepsis and septic shock in adult and paediatric patients. Cochrane Database Syst Rev. (2012). 12(12):CD004388. doi: 10.1002 / 14651858.CD004388.pub6 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
75. Meara CHO, Coupland LA, Kordbacheh F, et al. Neutralizing the pathological effects of extracellular histones with small polyanions. Nat Commun. (2020). 11(1):6408. doi:10.1038/s41467-020-20231-y [PMC free article] [PubMed] [CrossRef] [Google Scholar]
76. Boone BA, Murthy P, Miller-Ocuin J, Doerfler WR, Ellis JT, Liang X, et al. Chloroquine reduces hypercoagulability in pancreatic cancer through inhibition of neutrophil extracellular traps. BMC Cancer. (2018) 18:678. doi: 10.1186/s12885-018-4584-2 [PubMed Abstract] [CrossRef Full Text] [Google Scholar]
77. Caudrillier A, Kessenbrock K, Gilliss BM, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. The Journal of Clinical Investigation. (2012). 122(7):2661-2671. doi: 10.1172 / JCI61303 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
78. Liverani E, Rico MC, Tsygankov AY, Kilpatrick LE, Kunapuli SP. P2Y12 Receptor Modulates Sepsis-Induced Inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology. (2016). 36(5):961-971. doi: 10.1161 / ATVBAHA.116.307401 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
79. Mansour A, Bachelot-Loza C, Nesseler N, Gaussem P, Gouin-Thibault I. P2Y12 Inhibition beyond Thrombosis: Effects on Inflammation. Int J Mol Sci. 2020;21(4):1391. doi:10.3390/ijms21041391 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
80. Eisen D. P., Reid D., McBryde E. S. Acetyl salicylic acid usage and mortality in critically ill patients with the systemic inflammatory response syndrome and sepsis. Critical Care Medicine. (2012). 40(6):1761–1767. doi: 10.1097/CCM.0b013e318246b9df [PubMed] [CrossRef] [Google Scholar]
81. Gollomp K, Sarkar A, Harikumar S, et al. Fc-modified HIT-like monoclonal antibody as a novel treatment for sepsis. Blood. (2020). 135(10):743-754. doi: 10.1182/blood.2019002329 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
82. Shen X, Cao K, Zhao Y, Du J. Targeting Neutrophils in Sepsis: From Mechanism to Translation. Front Pharmacol. 2021;12:644270. doi: 10.3389/fphar.2021.644270 [PMC free article]