European Review for Medical and Pharmacological Sciences 2023; 27: 9559-9568

NETs, infections, and antimicrobials: a complex interplay

T.Z. ARABI[1], N.A. FAWZY[1], S. ABDUL RAB[1], G. ALABDUL RAZZAK[1], B.N. SABBAH[1], K. ALKATTAN[1], I. TLEYJEH[2-4], A. YAQINUDDIN[1]

[1]College of Medicine, Alfaisal University, Riyadh, Saudi Arabia
[2]Department of Medical Specialties, Infectious Diseases Section, King Fahad Medical City, Riyadh, Saudi Arabia
[3]Division of Infectious Diseases, Mayo Clinic College of Medicine and Science, Rochester, MN, United States
[4]Division of Epidemiology, Mayo Clinic College of Medicine and Science, Rochester, MN, United States

Abstract. – In response to a range of stimuli, neutrophils produce web-like structures known as neutrophil extracellular traps (NETs). The benefits of NETs in pathogen control are commonly offset by excessive release as part of a pro-inflammatory response, as shown in several disorders. The discovery of potential drugs that regulate NET release has helped to enhance our understanding of the role of NETs in immunological protection, inflammatory diseases, and autoimmune disorders. Emerging evidence has indicated that antimicrobials play an immunomodulatory role by influencing the levels of circulating NETs. Herein, we address NETosis in several disorders and detail the mechanisms of NET-mediated damage in infections. We also aim to evaluate recent evidence on the effects of antimicrobials on NET levels. Relevant keywords were searched in PubMed. Studies were evaluated for their relevance, and a narrative review was written accordingly. Several antibiotics, including beta-lactams and cephalosporins, alter NET formation and degradation in a protective manner, resulting in minimal host organ damage. Additionally, some studies have highlighted the immunomodulatory effects of antivirals and antifungals on NET. Further studies are needed to fully understand the clinical implications of NET-antimicrobial interactions and their underlying mechanisms.

Key Words:
Neutrophil extracellular traps, Infections, Antibiotics, Neutrophils, Immunomodulation, NET interactions.

Introduction

Neutrophils are the first immune cells to be recruited in sites of acute microbial infection, where they perform their functions by identifying, phagocytosing, and eliminating extracellular pathogens[1]. Other neutrophil functions include the production of reactive oxygen species (ROS), chemokine release, and a recently discovered function: the production of neutrophil extracellular traps (NETs). First identified by Brinkmann et al[2], NETs are extracellular, web-like structures that consist of decondensed neutrophil DNA, histones, and granular proteins.

It has been established[2] that NETs play a vital role in targeting microbes, preventing the dissemination of microbial infection, and colocalizing pathogens with highly toxic antimicrobial substances to enhance their destruction. The role of NETs, however, is not limited to microbial infections, nor is it always protective. Aberrant and massive production of NETs has been implicated in the pathogenesis of several diseases, such as SARS-CoV-2, diabetes, neurologic disorders, chronic obstructive pulmonary disease, cystic fibrosis, and several others[3-7]. Indeed, the pathological production of NETs is the driving force, and often the mediator, of damage in these conditions.

Antimicrobials play a significant role in host defense against various pathogens[8]. Several antimicrobials demonstrate immunomodulatory effects; however, the mechanisms underlying these effects are not well understood. Given the infamous tendency of NETs to cause extensive damage in other diseases, the existing literature on their possible pathological role in microbial infections and the complex interplay between antibiotics and NETs is limited. In this review, we survey the literature on the role of NETs in bacterial, fungal, and viral infections, as well as the possible interactions between antimicrobials and NETs.

Corresponding Author: Imad M. Tleyjeh, MD; e-mail: Tleyjeh.Imad@mayo.edu

Methods

The MEDLINE/PubMed database was searched for relevant keywords, including "neutrophil extracellular traps", "NETs", "antibiotics", "antifungals", and "antivirals". The resulting search was retrieved, and articles were reviewed. We included articles based on their preclinical and clinical relevance to the topic. Then, a narrative review article was written, describing the available literature surrounding the complex interactions between NETs, infections, and antimicrobials.

Mechanism of NET Formation

Once tissue injury ensues, damage-associated molecular patterns and pathogen-associated molecular patterns bind to pattern recognition receptors (PRRs) expressed at the surfaces of host cells, attracting neutrophils and other leukocytes to the site of action[9]. At the site of injury, neutrophils exert their effects by engulfing pathogens, releasing cytokines, producing reactive oxygen species (ROS), and generating NETs[10-12]. The mechanisms of NET formation are classified according to their dependence on the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme (Nox) for NET release[13] (Figure 1). In Nox-dependent pathways, neutrophil activation occurs *via* microbial products or phorbol 12-myristate 13-acetate (PMA), a potent chemical used to activate neutrophils *in vitro*. Additionally, studies[14] have also demonstrated that nanoparticles and molecular structures, such as polyhedral oligomeric silsesquioxanes, are capable of directly inducing NET formation in a dose-dependent matter. Then, neutrophils release calcium from intracellular stores, subsequently activating protein kinase C (PKC) and phosphorylating gp-91phox/Nox2[15]. As a result of these molecular changes, protein arginine deiminase 4 (PAD4) binds to intracellular calcium and enhances ROS production. The newly formed ROS causes cellular damage and, eventually, cell death[16]. Membrane damage causes granular components, such as neutrophil elastases (NE) and myeloperoxidases (MPO), to directly mix with nuclear chromatin and facilitate the process of decondensation. The 'decorated' chromatin is then free to be released out of the cell and promotes the extracellular antimicrobial functions of NETs (Figure 1)[17].

A study by Parker et al[18] initially suggested that NETs can be induced without the participation of Nox. *In vitro* administration of calcium ionophores revealed an elevated number of intracellular PAD4-calcium bindings. Ultimately, PAD4 translocates into the nucleus, leading to chromatin decondensation in a Nox-independent manner[19]. Additionally, several studies[20,21] demonstrated that Nox-independent NET formation can be further provoked *via* the activation of calcium-activated potassium channels of small conductance and mitochondrial ROS (mROS). mROS was originally thought to have no role in the production of ATP in neutrophils. Recently, however, it has been

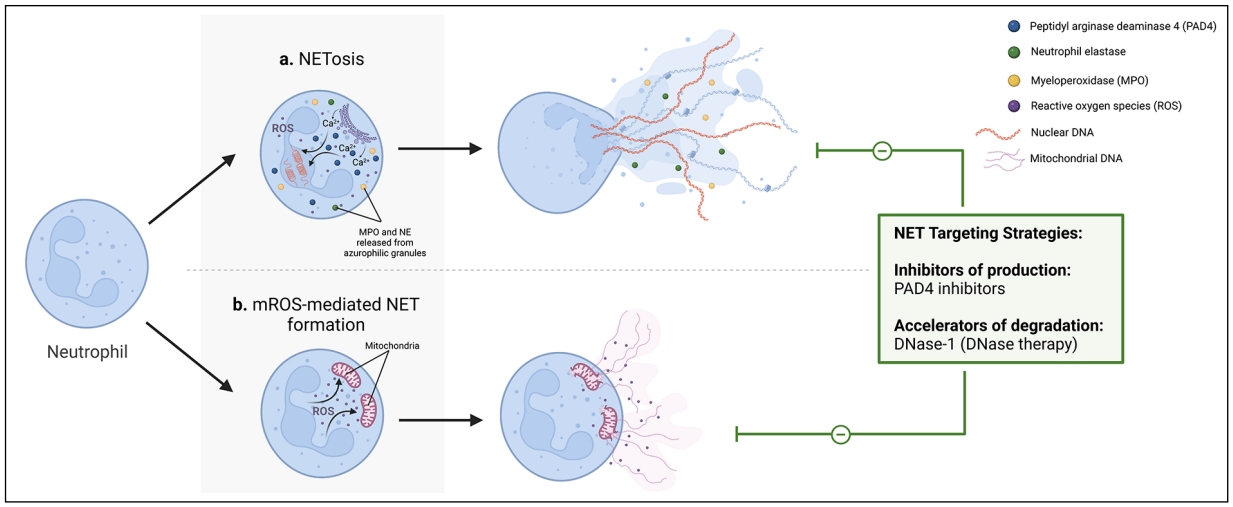

Figure 1. A diagram demonstrating the various pathways of NET formation: (**a**) represents NETosis, which involves apoptosis of the activated neutrophils, (**b**) shows how NETs can be generated alternatively through mROS-mediated pathways. Therapeutic strategies targeting NETs include PAD4 inhibitors, which inhibit NET production, and enhancers of degradation through DNase. This figure was made using BioRender.

associated with exacerbated immune responses and the induction of systemic diseases[22].

NETs Damage Tissues

It has been previously demonstrated[23] that NETs induce epithelial and endothelial damage in a dose-dependent manner. Histones, which account for 70% of NET proteins, and MPO seem to play significant roles as mediators of these cytotoxic effects[24,25]. Saffarzadeh et al[23] found that inhibiting histones and MPO results in a great reduction of NET-mediated cytotoxicity. Similarly, Xu et al[25] demonstrated that histone-inhibiting antibodies reduce mortality in mouse models of sepsis. Histones may be a potential therapeutic target in patients in pro-inflammatory states. Other components of NETs, such as human neutrophil peptides 1-3 and LL-37, also result in endothelial and bronchial epithelial cell death by inducing DNA breaks and cytochrome c release[26]. Large complexes also form between NET DNA threads and mucin in small bronchi, causing obstruction and facilitating further microbial growth within the airways[27]. Additionally, MPO and NE degrade glycosaminoglycan heparin sulfate, an essential component of lung tissue, which allows for enhanced neutrophil extravasation and an exacerbated immune response[27]. NE also decreases ciliary speed and damages endothelial actin cytoskeletons, E-cadherin, and VE-cadherin[27]. Collectively, these mechanisms are key players in NET-mediated endothelial and epithelial damage.

NETs also induce thrombosis by enhancing endothelial damage and activating complement systems[28]. Tumor necrosis factor-α, interleukin (IL)-4, and lipopolysaccharide (LPS) stimulate endothelial P-selectin secretion, which aggregates neutrophils and stimulates NET release[27,29]. Subsequently, NE consumes a tissue factor pathway inhibitor, promoting a pro-coagulation state[30]. Interestingly, inhibiting toll-like receptor (TLR)-2 and -4 significantly reduces the pro-coagulation effects of the NET-platelet axis, revealing that TLRs are key mediators of this pathway[31]. NETs also promote vessel inflammation, which is indicated by histological images of NET-containing vessels inflicted with vasculitis in COVID-19 hamster models[28]. A therapeutic strategy targeting NETs may prevent thrombosis, vasculitis, and damage to the epithelium and endothelium.

NETs and Bacteria

NETs possess antibacterial capabilities due to histones, cathepsin G, NE, MPO, lactoferrin, antimicrobial peptide LL-37, pentraxin 3, gelatinase, proteinase 3, and peptidoglycan-binding proteins[32-34]. Accordingly, NETs restrict or destroy bacterial development, including *Shigella flexneri, Pseudomonas aeruginosa, Escherichia coli, Shigella sonnei, Salmonella enteritidis, Salmonella typhimurium, Klebsiella pneumoniae,* and *Staphylococcus*[2,35]. Whole bacteria, as well as cell surface components of gram-positive and gram-negative bacteria, are potent stimuli of NETosis[36]. LPS, a significant component of the outer membrane of gram-negative bacteria, has been observed to stimulate NET formation[2]. It was further demonstrated that neutrophils can distinguish between LPS from various bacterial origins and selectively release NETs[37]. Under serum- and platelet-free conditions, neutrophils generated NETs in response to two of the seven distinct LPS structures examined, with LPS derived from *P. aeruginosa* (LPS-PA) proving to be particularly powerful. The ability of LPS-palmitic acid to induce NET release may be explained by the fact that P. aeruginosa is well-known for its tactics to evade phagocytosis, such as biofilm production[38]. Likewise, LPS from *E. coli* (serotype O128:B12; LPS-O128) produced NETosis in similar tissue conditions. Interestingly, however, four additional *E. coli* LPS serotypes (serotypes O55:B5, O127:B8, O111:B4, and O26:B6) that were also examined did not trigger NET release[37]. As a result, LPS-induced NETosis is not only species-specific but also serotype-specific[39,40].

NETs and Viruses

NETs were first recognized in viral infections by Narasaraju et al[41] in a mouse model of influenza pneumonitis. Viruses induce NET formation through a variety of mechanisms. Firstly, viruses can engage different PRRs, activating antiviral mechanisms in neutrophils[42]. Virus-infected cells also release many cytokines, such as IL-8 and type-1 interferon, which prepare neutrophils to release antiviral NETs[42-44]. Additionally, the influenza A virus directly stimulates NET formation; however, the detailed mechanisms of this are not yet well understood[42].

In COVID-19, the virus binds to angiotensin-converting enzyme 2 and causes excessive production of cytokines (also known as a cytokine storm)[45]. These cytokines further recruit inflammatory cells by activating PRRs, amplifying the inflammatory response[45]. Specifically, pyrin domain-containing protein 3 (NLRP3) activates the precursors of the cytokines IL-1β and IL-18. The NLRP3-produced IL-1β then recruits and activates neutrophils, inducing the release of NETs[46].

NETs and Fungi

NETs also play a major role in host defense against fungal infections. *Candida albicans, Aspergillus fumatigus, Histoplasma capsulatum, Phialophora verrucose, Paracoccidioides brasiliensis,* and *Scedosporium apiospermum* are some of the fungal species that have been described to induce NET formation[47]. The mechanisms of NET formation in fungal infections largely depend on the infectious organism. Several components of fungi, such as glucuronoxylomannogalactan, dectin-2, aspartic proteases, mannans, β-glucans, and farnesol, can directly activate neutrophils and induce a cascade of NET formation in human neutrophils[47]. Interestingly, *C. albicans* biofilms inhibit NET release and reduce fungal killing *in vitro* and animal models *via* reduction of ROS production[48]. These mechanisms can provide a therapeutic target, as future drugs may be developed to enhance NET formation and reduce immune escape in fungal infections[47].

Antibiotics Affect NET Release and Degradation

One possible mechanism of antibiotics' immunomodulatory role is their ability to influence the degree of NET formation in the host. Indeed, different classes of antibiotics, such as β-lactams and macrolides, have demonstrated this effect on NETs (Figure 2). Although studies are limited in this regard, this section highlights the available literature surrounding the effects of antibiotics on NET formation.

β-lactams and NETs

β-lactams are the most widely used antibiotics in the world[49]. Several studies in the literature have described the role of β-lactams in NET modulation in acute and chronic settings. Bysterzycka et al[50] assessed the effect of amoxicillin on NET release in neutrophils from healthy human donors. The

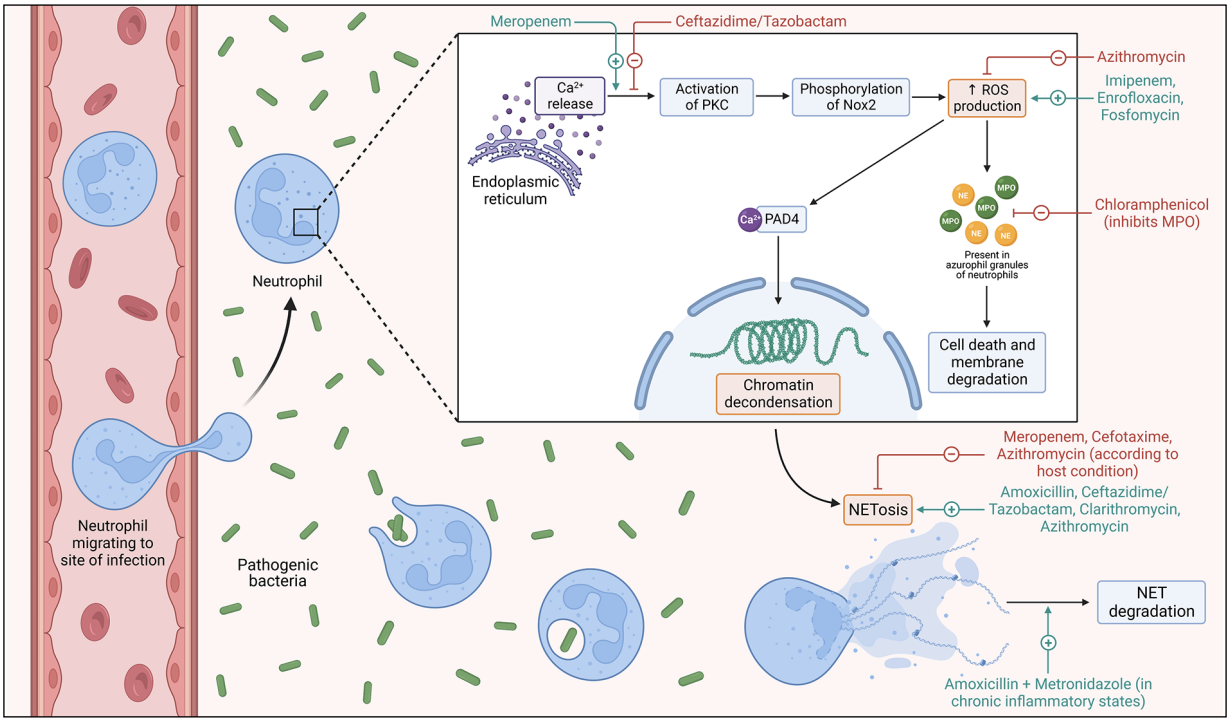

Figure 2. Antibiotics are able to decrease or increase NET levels *via* modulation of various steps of the NET pathways. This figure was made using BioRender.

authors found that amoxicillin significantly increased NET release in neutrophils stimulated by PMA. Incubation with amoxicillin also resulted in increased phagocytosis of adjacent *E. coli*, demonstrating the enhancing effects of amoxicillin on neutrophils. Contrarily, studies have found that amoxicillin downregulates NET production in chronic inflammatory states. For example, Moonen et al[51] demonstrated that periodontal therapy, amoxicillin and metronidazole, significantly increased NET degradation in chronic periodontitis patients at 3 months, 6 months, and 9 months post-treatment.

Xie et al[52] also studied the immunomodulatory effects of meropenem and ceftazidime/tazobactam on NET formation, ROS generation, and Nox activity. Meropenem slightly reduced NETosis, while ceftazidime/tazobactam significantly increased NETosis in PMA-activated human neutrophils but not in resting neutrophils. Both biotics reduced ROS production and NADPH in activated and resting neutrophils. Overall, the study[52] demonstrated that meropenem and ceftazidime/tazobactam modulate ROS-derived NET formation *via* the protein kinase C (PKC)-protein kinase B (Akt)-mammalian TOR (mTOR) pathway. Contrarily, imipenem, a carbapenem similar to meropenem, has been shown[53] to promote NET formation through a ROS-dependent mechanism in activated mouse neutrophils. The varying results may be due to the differences in the studied species; however, this theory has not been validated yet.

The effects of cefotaxime, a third-generation cephalosporin similar to ceftazidime, on NETs in human neutrophils have also been studied. Unlike ceftazidime, cefotaxime shows no effect on NET release, as discovered by Manda-Handzlik et al[54]. Duan et al[53], on the other hand, demonstrated that cefotaxime inhibits NET release in activated mouse neutrophils through a ROS-dependent mechanism. The mechanisms underlying the varying effects of ceftazidime and cefotaxime on NETs remain unclear. The differences may be attributed to the combination of ceftazidime with tazobactam, an inhibitor of β-lactamase, studied by Xie et al[52], instead of the standalone third-generation cephalosporin studied by Manda-Handzlik et al[54] and Duan et al[53]. In mouse models[53] of sepsis, de-escalation therapy, defined as a switch from broad-spectrum imipenem to narrow-spectrum ceftriaxone, promotes NET formation in early sepsis and inhibits NET formation in the late stages of sepsis. Escalation therapy (the opposite of de-escalation therapy) results in contrasting effects compared to its counterpart[53]. Overall, β-lactams have mostly been found to mediate protective immunomodulatory effects.

Macrolides and NETs

The addition of macrolides to the standard treatment regimen of pneumonia secondary to macrolide-resistant organisms has significantly reduced the mortality of pneumonia patients, demonstrating a possible immunomodulatory role of macrolides[55]. Several studies in the literature have elucidated the effects of macrolides on NET formation. For example, Konstantinidis et al56 demonstrated that clarithromycin and azithromycin increased NET formation in healthy human neutrophils *in vitro*. The authors found that clarithromycin induced the formation of LL-37-bearing NETs in *Helicobacter pylori*-positive gastritis patients *via* the autophagy-dependent pathway. Furthermore, clarithromycin-induced NETs reduce the growth of *Acinetobacter baumannii* and the production of biofilm through the expression of LL-37. It is worth noting that PMA-induced NETs do not demonstrate any effect on the generation of *A. baumannii* biofilm[56].

LL-37, the active form of human cathelicidin, plays a crucial role in the defense against bacterial and fungal infections[57]. LL-37 may also be involved in wound healing by enhancing vascularization[58]. Arampatzioglou et al[58] studied the effects of clarithromycin and LL-37-bearing NETs on antibacterial activity and wound healing in type 2 diabetes mellitus (T2DM) patients. NETs of T2DM patients lacked antibacterial activity; however, this effect was reversed by clarithromycin *via* the production of LL-37-expressing NETs in well-controlled T2DM neutrophils *in vitro*. Furthermore, the authors demonstrated that clarithromycin-induced NETs enhance wound healing in T2DM by activating and promoting the differentiation of skin fibroblasts. Overall, these results provide further insight into the immunomodulatory effects of clarithromycin and the important role of LL-37 in antimicrobial activities and promoting wound healing.

Interestingly, erythromycin and azithromycin seem to have opposing effects on NETs compared to clarithromycin. In mouse models[59] of emphysema, erythromycin decreases NET levels and ameliorates emphysema by downregulating Th1 and Th17 cells and suppressing myeloid dendritic cells, demonstrating a possible therapeutic indication for erythromycin in emphysema patients. Similarly, azithromycin prevents ROS production and cell degranulation and significantly reduces

NET formation in healthy human neutrophils[60]. Hence, macrolides may be able to influence the release of NETs according to the host's needs.

Other antibiotics, such as enrofloxacin and gentamicin, also influence the process of NET formation. These findings have been summarized in Table I. Studies exploring the effects of antibiotics on NETs in human subjects remain limited. Nonetheless, future preclinical research may yield promising results in employing the complex interplay of antibiotics and NETs as a therapeutic mechanism.

Antivirals, Antifungals, and NETs

There are few studies assessing the effects of antivirals and antifungals on NET formation and degradation. Ashar et al[61] found that treatment of influenza-treated mice with a combination of oseltamivir and a CXC chemokine receptor 2 antagonist improved survival and reduced NET release. Notably, the combination of both drugs had greater effects than either drug alone. Similarly, the combination attenuated lung pathology in piglets with sublethal swine influenza[61].

Echinocandin treatment of *C. albicans* biofilms has also been shown[62] to promote NET release by up to 8-fold compared to non-treated neutrophils. This finding may explain the synergistic anti-*C. albicans* effects seen between phagocytes and echinocandins[63]. Further studies are needed to explore the immunomodulatory roles of antivirals and antifungals and the mechanisms underlying these roles.

Clinical Implications

Overall, preclinical studies have demonstrated that antibiotics influence NET levels in a protective manner, regardless of increases or decreases in such levels. De-escalation therapy results in decreased lung, liver, and intestine damage in mouse models[53] of sepsis by modulating NET release in the early and late stages of sepsis. Zhu et al[64] found that dexamethasone reduced neutrophil infiltration, NETs, and bacterial burden in mouse models of *P. aeruginosa keratitis*. Additionally, TobraDex (a combination of tobramycin and dexamethasone) reduced these parameters to a greater extent than dexamethasone[64]. Accordingly, TobraDex-treated mice exhibited slight corneal damage, while dexamethasone-treated mice suffered severe corneal damage[64]. These data indicate that the antibiotic-mediated effects on NETs influence the severity of organ damage in bacterial infections.

Clinical studies assessing the interactions between antibiotics and NETs remain limited. An international, multicohort study by Keir et al[65] assessed NET levels as markers of response to antibiotics in bronchiectasis and asthmatic patients. The authors showed that bronchiectasis patients with the highest

Table I. A summary of the various effects of antibiotics on NETs.

Class	Subclass	Antibiotic	Sample type	Effect on NET activities	Reference
β-lactams	Penicillins	Amoxicillin	Healthy humans, chronic periodontitis patients	↑ NET release, ↑ degradation	50, 51
	Cephalosporins	Ceftazidime	Healthy humans	↓ ROS	52
		Cefotaxime	Healthy humans, mice	No effect on NET release in humans, ↓ release in mice	53, 54
	Carbapenems	Meropenem	Healthy humans	↓ ROS	52
		Imipenem	Mice	↑ ROS	53
Macrolides		Clarithromycin	Healthy humans, T2DM patients	↑ NET formation	56, 58
		Azithromycin	Healthy humans	↑ NET formation, ↓ ROS and NET formation	58, 60, 66
		Erythromycin	Mice	↓ ROS and NET formation	59
Fluoroquinolones		Enrofloxacin	Bovines	↑ NET formation	67
Nitroimidazoles		Metronidazole	Chronic periodontitis patients	↑ NET degradation	51
Phosphonic acids		Fosfomycin	Mice	↑ ROS and NET formation	68
Aminoglycosides		Gentamicin	Healthy humans	↓ NET release	54
Lincomycins		Clindamycin	Healthy humans	No effect on NET release	50
Glucocorticoids		Methyl-prednisolone	Canines	↑ NET formation	69
Chloramphenicol			Healthy humans	↓ NET release	60

NET levels had more exacerbations, a shorter time to severe exacerbations, and increased mortality[65]. Low-dose azithromycin resulted in decreased NET sputum concentrations over 12 months in both bronchiectasis and asthma patients[65]. Understanding the mechanisms behind antimicrobial-NET interactions may allow physicians to provide tailored therapy targeting various immune functions of the host. Future clinical studies are needed to fully assess the value of NETs as markers of prognosis and response to antibiotic treatment.

Conclusions

Modulating NETs is quickly becoming a therapeutic focus in the treatment of a variety of disease processes. Antibiotics may demonstrate a protective role in reducing organ damage *via* NET modulation. Clinically, NETs may provide the basis for future prognostic and therapeutic markers in patients with acute and chronic infections. Additionally, antibiotics have been noted to improve wound healing by influencing NET levels in diabetic patients.

Overall, the complex interaction between antibiotics and NETs seems to provide support tailored to the needs of the host, increasing microbial killing, improving wound healing, and reducing organ damage. Targeting NETs in a therapeutic manner may open the door for a multitude of opportunities in the treatment of viral, fungal, and bacterial infections. More research must be undertaken to examine the mode of action of these effects and their effectiveness in clinical, real-life situations.

Funding
None.

Authors' Contributions
Conceptualization: T.Z.A., I.T., and A.Y..; writing-original draft preparation: T.Z.A., N.A.F., G.A.R., S.A.R., B.N.S., K.A., I.T., and A.Y.; writing-review and editing: T.Z.A., N.A.F., I.T., and A.Y.; supervision: I.T. and A.Y.; project administration: K.A., I.T., and A.Y.; All authors have read and agreed to the published version of the manuscript.

ORCID ID
Tarek Ziad Arabi: 0000-0002-6292-7683
Nader Ashraf Fawzy: 0000-0002-4096-6139
Saleha Abdul Rab: 0000-0003-4179-927X
Ghazi Alabdul Razzak: 0009-0008-7160-6524
Belal Nedal Sabbah: 0000-0001-9131-7430
Khaled Alkattan: 0000-0002-1372-9883
Imad Tleyjeh: 0000-0002-5551-0353
Ahmed Yaqinuddin: 0000-0001-7536-910X.

Conflicts of Interest
The authors declare that they have no conflict of interest to declare.

Data Availability
Data sharing is not applicable to this manuscript, as no data were generated in this study.

Informed Consent
Not applicable.

Ethics Approval
Not applicable.

References

1) Malech HL, Deleo FR, Quinn MT. The Role of Neutrophils in the Immune System: An Overview. Humana Press, 2014.
2) Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil Extracellular Traps Kill Bacteria. Science 2004; 303: 1532-1535.
3) Pezeshki S. Neutrophil Extracellular Traps (NET) and SARS-CoV-2. Immunopharmacol Immunotoxicol 2023; 45: 253-255.
4) Shafqat A, Abdul Rab S, Ammar O, Al Salameh S, Alkhudairi A, Kashir J, Alkattan K, Yaqinuddin A. Emerging role of neutrophil extracellular traps in the complications of diabetes mellitus. Front Med (Lausanne) 2022; 9: 995993.
5) Vaibhav K, Braun M, Alverson K, Khodadadi H, Kutiyanawalla A, Ward A, Banerjee C, Sparks T, Malik A, Rashid MH, Khan MB, Waters MF, Hess DC, Arbab AS, Vender JR, Hoda N, Baban B, Dhandapani KM. Neutrophil extracellular traps exacerbate neurological deficits after traumatic brain injury. Sci Adv 2020; 6: eaax8847.
6) Tsagkaris C, Bilal M, Aktar I, Aboufandi Y, Tas A, Aborode AT, Suvvari TK, Ahmad S, Shkodina A, Phadke R, Emhamed MS, Baig AA, Alexiou A, Ashraf GM, Kamal MA. Cytokine storm and neuropathological alterations in patients with neurological manifestations of COVID-19. Curr Alzheimer Res 2022.
7) Trivedi A, Khan MA, Bade G, Talwar A. Orchestration of Neutrophil Extracellular Traps (Nets), a Unique Innate Immune Function during Chronic Obstructive Pulmonary Disease (COPD) Development. Biomedicines 2021; 9: 53.

8) Bastida C, Soy D, Torres A. The safety of antimicrobials for the treatment of community-acquired pneumonia. Expert Opin Drug Saf 2020; 19: 577-587.
9) Denning NL, Aziz M, Gurien SD, Wang P. DAMPs and NETs in Sepsis. Front Immunol 2019; 10: 2536.
10) Scapini P, Cassatella MA. Social networking of human neutrophils within the immune system. Blood 2014; 124: 710-719.
11) Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: from mechanisms to disease. Annu Rev Immunol 2012; 30: 459-489.
12) Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol 2011; 11: 519-531.
13) Tatsiy O, McDonald PP. Physiological Stimuli Induce PAD4-Dependent, ROS-Independent NETosis, With Early and Late Events Controlled by Discrete Signaling Pathways. Front Immunol 2018; 9: 2036.
14) Rizzi M, Carniato F, Tonello S, Migliario M, Invernizzi M, Rocchetti V, Marchese L, Renò F. Charged molecular silica trigger in vitro NETosis in human granulocytes via both oxidative and autophagic pathways. Eur Rev Med Pharmacol Sci 2018; 22: 7058-7068.
15) Kaplan MJ, Radic M. Neutrophil Extracellular Traps: Double-Edged Swords of Innate Immunity. J Immunol 2012; 189: 2689-2695.
16) Khan MA, Farahvash A, Douda DN, Licht J-C, Grasemann H, Sweezey N, Palaniyar N. JNK Activation Turns on LPS- and Gram-Negative Bacteria-Induced NADPH Oxidase-Dependent Suicidal NETosis. Sci Rep 2017; 7: 3409.
17) Ravindran M, Khan MA, Palaniyar N. Neutrophil Extracellular Trap Formation: Physiology, Pathology, and Pharmacology. Biomolecules 2019; 9: 365.
18) Parker H, Dragunow M, Hampton MB, Kettle AJ, Winterbourn CC. Requirements for NADPH oxidase and myeloperoxidase in neutrophil extracellular trap formation differ depending on the stimulus. J Leukoc Biol 2012; 92: 841-849.
19) Wang Y, Li M, Stadler S, Correll S, Li P, Wang D, Hayama R, Leonelli L, Han H, Grigoryev SA, Allis CD, Coonrod SA. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. J Cell Biol 2009; 184: 205-213.
20) Maianski NA, Geissler J, Srinivasula SM, Alnemri ES, Roos D, Kuijpers TW. Functional characterization of mitochondria in neutrophils: a role restricted to apoptosis. Cell Death Differ 2004; 11: 143-153.
21) Fay AJ, Qian X, Jan YN, Jan LY. SK channels mediate NADPH oxidase-independent reactive oxygen species production and apoptosis in granulocytes. Proc Natl Acad Sci U S A 2006; 103: 17548-17553.
22) Douda DN, Khan MA, Grasemann H, Palaniyar N. SK3 channel and mitochondrial ROS mediate NADPH oxidase-independent NETosis induced by calcium influx. Proc Natl Acad Sci U S A 2015; 112: 2817-2822.
23) Saffarzadeh M, Juenemann C, Queisser MA, Lochnit G, Barreto G, Galuska SP, Lohmeyer J, Preissner KT. Neutrophil Extracellular Traps Directly Induce Epithelial and Endothelial Cell Death: A Predominant Role of Histones. PLoS One 2012; 7: e32366.
24) Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, Brinkmann V, Jungblut PR, Zychlinsky A. Neutrophil Extracellular Traps Contain Calprotectin, a Cytosolic Protein Complex Involved in Host Defense against Candida albicans. PLoS Path 2009; 5: e1000639.
25) Xu J, Zhang X, Pelayo R, Monestier M, Ammollo CT, Semeraro F, Taylor FB, Esmon NL, Lupu F, Esmon CT. Extracellular histones are major mediators of death in sepsis. Nat Med 2009; 15: 1318-1321.
26) Aarbiou J, Tjabringa GS, Verhoosel RM, Ninaber DK, White SR, Peltenburg LTC, Rabe KF, Hiemstra PS. Mechanisms of cell death induced by the neutrophil antimicrobial peptides α-defensins and LL-37. Inflamm Res 2006; 55: 119-127.
27) Szturmowicz M, Demkow U. Neutrophil Extracellular Traps (NETs) in Severe SARS-CoV-2 Lung Disease. Int J Mol Sci 2021; 22: 8854.
28) Becker K, Beythien G, de Buhr N, Stanelle-Bertram S, Tuku B, Kouassi NM, Beck S, Zickler M, Allnoch L, Gabriel G, von Köckritz-Blickwede M, Baumgärtner W. Vasculitis and Neutrophil Extracellular Traps in Lungs of Golden Syrian Hamsters With SARS-CoV-2. Front Immunol 2021; 12: 640842.
29) Fard MB, Fard SB, Ramazi S, Atashi A, Eslamifar Z. Thrombosis in COVID-19 infection: Role of platelet activation-mediated immunity. Thromb J 2021; 19: 59.
30) Massberg S, Grahl L, von Bruehl ML, Manukyan D, Pfeiler S, Goosmann C, Brinkmann V, Lorenz M, Bidzhekov K, Khandagale AB, Konrad I, Kennerknecht E, Reges K, Holdenrieder S, Braun S, Reinhardt C, Spannagl M, Preissner KT, Engelmann B. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. Nat Med 2010; 16: 887-896.
31) Semeraro F, Ammollo CT, Morrissey JH, Dale GL, 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.
32) Brinkmann V, Zychlinsky A. Neutrophil extracellular traps: is immunity the second function of chromatin? J Cell Biol 2012; 198: 773-783.
33) Neumann A, Berends ET, Nerlich A, Molhoek EM, Gallo RL, Meerloo T, Nizet V, Naim HY, von Köckritz-Blickwede M. The antimicrobial peptide LL-37 facilitates the formation of neutrophil extracellular traps. Biochem J 2014; 464: 3-11.
34) Burgener SS, Schroder K. Neutrophil Extracellular Traps in Host Defense. Cold Spring Harb Perspect Biol 2020; 12: a037028.

35) Delgado-Rizo V, Martínez-Guzmán MA, Iñiguez-Gutierrez L, García-Orozco A, Alvarado-Navarro A, Fafutis-Morris M. Neutrophil Extracellular Traps and Its Implications in Inflammation: An Overview. Front Immunol 2017; 8: 81.

36) Neeli I, Khan SN, Radic M. Histone Deimination As a Response to Inflammatory Stimuli in Neutrophils. J Immunol 2008; 180: 1895-1902.

37) Pieterse E, Rother N, Yanginlar C, Hilbrands LB, van der Vlag J. Neutrophils Discriminate between Lipopolysaccharides of Different Bacterial Sources and Selectively Release Neutrophil Extracellular Traps. Front Immunol 2016; 7: 484.

38) Alhede M, Bjarnsholt T, Givskov M, Alhede M. Pseudomonas aeruginosa biofilms: mechanisms of immune evasion. Adv Appl Microbiol 2014; 86: 1-40.

39) Migale R, Herbert BR, Lee YS, Sykes L, Waddington SN, Peebles D, Hagberg H, Johnson MR, Bennett PR, MacIntyre DA. Specific Lipopolysaccharide Serotypes Induce Differential Maternal and Neonatal Inflammatory Responses in a Murine Model of Preterm Labor. Am J Pathol 2015; 185: 2390-2401.

40) Eder K, Vizler C, Kusz E, Karcagi I, Glavinas H, Balogh GE, Vigh L, Duda E, Gyorfy Z. The role of lipopolysaccharide moieties in macrophage response to Escherichia coli. Biochem Biophys Res Commun 2009; 389: 46-51.

41) Narasaraju T, Yang E, Samy RP, Ng HH, Poh WP, Liew AA, Phoon MC, van Rooijen N, Chow VT. Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. Am J Pathol 2011; 179: 199-210.

42) Schönrich G, Raftery MJ. Neutrophil Extracellular Traps Go Viral. Front Immunol 2016; 7: 366.

43) Martinelli S, Urosevic M, Daryadel A, Oberholzer PA, Baumann C, Fey MF, Dummer R, Simon HU, Yousefi S. Induction of Genes Mediating Interferon-dependent Extracellular Trap Formation during Neutrophil Differentiation. J Biol Chem 2004; 279: 44123-44132.

44) Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of a human respiratory epithelial cell line with rhinovirus. Induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. J Clin Invest 1995; 96: 549-557.

45) Janiuk K, Jabłońska E, Garley M. Significance of NETs Formation in COVID-19. Cells 2021; 10: 151.

46) Yaqinuddin A, Kashir J. Novel therapeutic targets for SARS-CoV-2-induced acute lung injury: Targeting a potential IL-1β/neutrophil extracellular traps feedback loop. Med Hypotheses 2020; 143: 109906.

47) Zhong H, Lu RY, Wang Y. Neutrophil extracellular traps in fungal infections: A seesaw battle in hosts. Front Immunol 2022; 13: 977493.

48) Johnson CJ, Cabezas-Olcoz J, Kernien JF, Wang SX, Beebe DJ, Huttenlocher A, Ansari H, Nett JE. The Extracellular Matrix of Candida albicans Biofilms Impairs Formation of Neutrophil Extracellular Traps. PLoS Path 2016; 12: e1005884.

49) Bush K, Bradford PA. β-Lactams and β-Lactamase Inhibitors: An Overview. Cold Spring Harb Perspect Med 2016; 6: a025247.

50) Bystrzycka W, Moskalik A, Sieczkowska S, Manda-Handzlik A, Demkow U, Ciepiela O. The effect of clindamycin and amoxicillin on neutrophil extracellular trap (NET) release. Cent Eur J Immunol 2016; 41: 1-5.

51) Moonen CG, Buurma KG, Faruque MR, Balta MG, Liefferink E, Bizzarro S, Nicu EA, Loos BG. Periodontal therapy increases neutrophil extracellular trap degradation. Innate Immun 2020; 26: 331-340.

52) Xie T, Duan Z, Sun S, Chu C, Ding W. β-Lactams modulate neutrophil extracellular traps formation mediated by mTOR signaling pathway. Biochem Biophys Res Commun 2021; 534: 408-414.

53) Duan Z, Xie T, Chu C, Chen F, Wang X, Li J, Ding W. De-escalation antibiotic therapy alleviates organ injury through modulation of NETs formation during sepsis. Cell Death Discov 2021; 7: 345.

54) Manda-Handzlik A, Bystrzycka W, Sieczkowska S, Demkow U, Ciepiela O. Antibiotics Modulate the Ability of Neutrophils to Release Neutrophil Extracellular Traps. Springer International Publishing, 2016.

55) Restrepo MI, Mortensen EM, Waterer GW, Wunderink RG, Coalson JJ, Anzueto A. Impact of macrolide therapy on mortality for patients with severe sepsis due to pneumonia. Eur Respir J 2009; 33: 153-159.

56) Konstantinidis T, Kambas K, Mitsios A, Panopoulou M, Tsironidou V, Dellaporta E, Kouklakis G, Arampatzioglou A, Angelidou I, Mitroulis I, Skendros P, Ritis K. Immunomodulatory Role of Clarithromycin in Acinetobacter baumannii Infection via Formation of Neutrophil Extracellular Traps. Antimicrob Agents Chemother 2016; 60: 1040-1048.

57) Kahlenberg JM, Kaplan MJ. Little Peptide, Big Effects: The Role of LL-37 in Inflammation and Autoimmune Disease. J Immunol 2013; 191: 4895-4901.

58) Arampatzioglou A, Papazoglou D, Konstantinidis T, Chrysanthopoulou A, Mitsios A, Angelidou I, Maroulakou I, Ritis K, Skendros P. Clarithromycin Enhances the Antibacterial Activity and Wound Healing Capacity in Type 2 Diabetes Mellitus by Increasing LL-37 Load on Neutrophil Extracellular Traps. Front Immunol 2018; 9: 2064.

59) Zhang H, Qiu SL, Tang QY, Zhou X, Zhang JQ, He ZY, Bai J, Li MH, Deng JM, Liang Y, Zhong XN. Erythromycin suppresses neutrophil extracellular traps in smoking-related chronic pulmonary inflammation. Cell Death Dis 2019; 10: 678.

60) Bystrzycka W, Manda-Handzlik A, Sieczkowska S, Moskalik A, Demkow U, Ciepiela O. Azithromycin and Chloramphenicol Diminish Neutrophil Extracellular Traps (NETs) Release. Int J Mol Sci 2017; 18: 2666.

61) Ashar HK, Pulavendran S, Rudd JM, Maram P, Achanta M, Chow VTK, Malayer JR, Snider TA, Teluguakula N. Administration of a CXC Chemokine Receptor 2 (CXCR2) Antagonist, SCH527123, Together with Oseltamivir Suppresses NETosis

and Protects Mice from Lethal Influenza and Piglets from Swine-Influenza Infection. Am J Pathol 2021; 191: 669-685.

62) Hoyer AR, Johnson CJ, Hoyer MR, Kernien JF, Nett JE. Echinocandin Treatment of Candida albicans Biofilms Enhances Neutrophil Extracellular Trap Formation. Antimicrob Agents Chemother 2018; 62: e00797-18.

63) Katragkou A, Kruhlak MJ, Simitsopoulou M, Chatzimoschou A, Taparkou A, Cotten CJ, Paliogianni F, Diza-Mataftsi E, Tsantali C, Walsh TJ, Roilides E. Interactions between Human Phagocytes and Candida albicans Biofilms Alone and in Combination with Antifungal Agents. J Infect Dis 2010; 201: 1941-1949.

64) Zhu B, Zhang L, Yuan K, Huang X, Hu R, Jin X. Neutrophil extracellular traps may have a dual role in Pseudomonas aeruginosa keratitis. Eur J Clin Microbiol Infect Dis 2021; 40: 169-180.

65) Keir HR, Shoemark A, Dicker AJ, Perea L, Pollock J, Giam YH, Suarez-Cuartin G, Crichton ML, Lonergan M, Oriano M, Cant E, Einarsson GG, Furrie E, Elborn JS, Fong CJ, Finch S, Rogers GB, Blasi F, Sibila O, Aliberti S, Simpson JL, Huang JTJ, Chalmers JD. Neutrophil extracellular traps, disease severity, and antibiotic response in bronchiectasis: an international, observational, multicohort study. Lancet Respir Med 2021; 9: 873-884.

66) Konstantinidis T, Kambas K, Mitsios A, Panopoulou M, Tsironidou V, Dellaporta E, Kouklakis G, Arampatzioglou A, Angelidou I, Mitroulis I, Skendros P, Ritis K. Immunomodulatory Role of Clarithromycin in Acinetobacter baumannii Infection via Formation of Neutrophil Extracellular Traps. Antimicrob Agents Chemother 2016; 60: 1040-1048.

67) Jerjomiceva N, Seri H, Völlger L, Wang Y, Zeitouni N, Naim HY, von Köckritz-Blickwede M. Enrofloxacin Enhances the Formation of Neutrophil Extracellular Traps in Bovine Granulocytes. J Innate Immun 2014; 6: 706-712.

68) Shen F, Tang X, Cheng W, Wang Y, Wang C, Shi X, An Y, Zhang Q, Liu M, Liu B, Yu L. Fosfomycin enhances phagocyte-mediated killing of Staphylococcus aureus by extracellular traps and reactive oxygen species. Sci Rep 2016; 6: 19262.

69) Steffensen N, Imker R, Lassnig S, Fulde M, Rieder JC, de Buhr N. Methylprednisolone Induces Extracellular Trap Formation and Enhances Bactericidal Effect of Canine Neutrophils. Int J Mol Sci 2021; 22: 7734.