

REVIEW

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# “In sickness and in health” – how neutrophil extracellular trap (NET) works in infections, selected diseases and pregnancy

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## Abstract

The discovery of the NET network (neutrophil extracellular trap) has revolutionized the perception of defense mechanisms used by neutrophils in infections and non-infectious states, as this mechanism proves the complexity of the ways in which neutrophils can act in the organism. The paper describes the NET network and its participation in bacterial, viral, fungal and parasitic infections, both in a positive and a negative aspect. In addition, attention was paid to the participation of NETs in the course of autoimmune diseases, cancer, as well as its impact on pregnancy and fertility in mammals.

**Keywords:** NET, neutrophil, immunology

## Introduction

Neutrophil granulocytes (neutrophils, PMN - polymorphonuclear leukocytes) play a major role in natural immunity. They are characterized by their ability to conduct or feature phagocytosis, pinocytosis, cytotoxicity and cytolysis and form the first line of defense in antimicrobial immunity [1–5]. A new function of neutrophil granulocytes, their ability to form so-called NETs (neutrophil extracellular traps) was discovered in 2004 [6]. Microorganisms are bound outside cells in a network that can be called ‘extracellular phagocytosis’. This new feature of PMN cells has changed the understanding of their defense mechanisms as they display activity not only intra- but also extracellularly.

NETs were first identified in mice and humans. Later on, the ability to form extracellular traps was confirmed in other mammals, including cattle, horses, cats, rabbits, birds, fish and invertebrates [7, 8]. More recently, the ability to form extracellular networks apart from mammals was found in eosinophils - EET (eosinophil extracellular trap) network [9], macrophages/monocytes - MET (macrophage/monocyte extracellular trap) network [10], mastocytes - MCET (mast cell extracellular

trap) network [11, 12], basophils - BET (basophil extracellular trap) network [13], heterophils in birds - HET (heterophil extracellular trap) network [14], phagocytes in fish [15] (mainly in carp’s neutrophils) and hemocytes in invertebrates, in marine decapods [16] and celomocytes in worms. There is no data in literature on existence of ETs in reptiles and amphibian, but there is possibility of finding such a structure in plants. Platelets are also involved in the formation of ETs (extracellular traps). When activated, they bind to neutrophils through TLR4 receptor and facilitate network formation [17, 18]. It was shown [19], that platelets, by aggregating to NET, influence its functioning and enlarge the trap with erythrocytes and other serum factors, like von Willebrand factor, fibronectin, fibrinogen, which stabilizes the NET.

There are data suggesting that respiratory burst and formation of ROS is crucial for NET formation. It was shown that NADPH oxidase-derived ROS influence NET formation [20], but also other sources of ROS have been described as potentially useful for NET formation, such as singlet oxygen [21] and HOCl and HOBr [22]. Nevertheless, ROS-independent NETosis also appears and the NET formation is induced by calcium influx [23]. Moreover, chromatin decondensation during NET formation may be caused by histone citrullination catalyzed by PAD4 (peptidylarginine deiminase 4) [24], but the role of histone deimination in the process of NET

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formation is still to be explored. There are also some studies showing that autophagy promotes formation of bacterial peptide N-formyl-Met-Leu-Phe - induced NET network [25], but the role of it is still debatable. Other factors that stimulate neutrophils into forming NETs are LPS, TNF $\alpha$ , IL-8, PAF, PMA [2], monosodium urate [8, 26], cholesterol [26, 27] and calcium carbonate crystals [26, 28].

NETs are formed of material from cell nucleus breakdown and neutrophil granules, including NE, MPO, cathepsin G, metalloproteinase-9, BPI protein, lactoferrin and gelatinase [6], however the core of the trap is constituted with chromatin with the diameter of 15-17 nm with protein domains with the diameter of 25 nm [2]. Moreover, histones from the cell nucleus (H1, H2A, H3 and H4) are 70% of the NET proteins [4]. Other constituent components include granular proteins, including proteinase 3, neutrophil serine proteases and cathelicidin (peptide LL-37) which in humans is indirectly implicated in killing microorganisms involved with NET network [3]. The peptide can extend the network life by providing protection against degradation by bacterial nucleases [3]. When a neutrophil is activated, a cascade of events occurs in effect resulting in cell breakdown. Inside the nucleus, decondensation of chromatin skeleton takes place, division into eu- and heterochromatin ceases to exist and after the rupture of the nuclear envelope stretches of DNA reach out into cytoplasm where nuclear histones and cytoplasmic granules and proteins are 'mixed'. The cell membrane breaks down too and the whole genetic material together with the mixture of mainly proteins is transported outside the cell. As a result of NET formation, neutrophils die in an isolated death mechanism, known as NETosis [1, 2, 4].

It has been recently described that this death can occur in two forms - as lytic NETosis, known also as suicidal NETosis and non-suicidal pathway, called vital NETosis. Conventional and frequently registered suicidal NETosis starts with ligand binding to neutrophil TLR and receptors for IgG-Fc, complement and cytokines, which causes calcium ions release to the cytoplasm. High level of calcium ions in cytoplasm induces ROS and under the influence of ROS cell membrane is destroyed and substances (NE and MPO) from granules migrate to nucleus, causing chromatin decondensation. This allows the release of NET but at the expense of cell life [29]. Contrary to suicidal NETosis, vital NETosis is a pathway, during which NET release occurs via blebbing of the nuclear envelope. It was reported that such pathway may be stimulated by LPS and appears rapidly, even 30 minutes after stimulation, comparing to hours for suicidal NETosis [30]. This kind of NETosis has been confirmed for processes induced by *Staphylococcus aureus* [31] and *Candida albicans* [32]. The only question raises whether neutrophils after vital NETosis should be termed "viable", since they lack parts of DNA.

### NETs in bacterial infections

Although there is an extensive body of reports on NET formation in bacterial infections, the way in which bacteria bind to the network strands remains not fully explained. One of the theses claims that the process relies on electrostatic interactions between negatively charged chromatin strands and positively charged surface of bacteria [18]. However, individual *Escherichia coli* or *Klebsiella pneumoniae* bacteria are thought to poorly induce neutrophil activation and NET formation [33]. *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) was used to demonstrate that only a larger amount of the bacteria can facilitate neutrophil extracellular trap release [33]. The lack of certain granules in NETs was shown to play a major role in the pathogenesis of some acute bacterial infections, since the composition of NETs translates into their efficacy [34]. A low concentration of cathepsin G in mice was found to increase their susceptibility to infection with Gram-positive bacteria which not having adequate levels of neutrophil elastase were more frequently infected with Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae* or other bacteria from the family *Enterobacteriaceae* [4]. DNA was also shown to be a NET microbicidal component and facilitate the killing of *Pseudomonas aeruginosa* [35]. The phenomenon was investigated *in vivo* in mice. After the addition of surplus Mg<sup>2+</sup> ions or alkaline phosphatase, DNA had the ability to chelate ions which is pivotal for NET microbicidal activity against *Pseudomonas aeruginosa*. The same components also inhibited production of components stabilizing the bacterial membrane and proteins protecting bacteria against NET inactivation. Therefore, DNA was concluded to contribute to antibacterial properties of NET and it also may serve as a signal to elicit host-resistance strategies [35]. Some antibiotics, including clindamycin and amoxicillin, may induce NET release into extracellular environment [36]. *Neisseria gonorrhoeae* infection, even though it causes delay in phagosome formation, also induces the formation of NETs [4]. NETs play an important role in systemic infection patients [37] and can affect them in two ways. NETs can use their microbicidal ability to control infection development, catch and destroy pathogens circulating in the blood. On the other hand though, NETs can facilitate tissue and organ damage. Furthermore, NETs degraded by recombinant human DNase promote bacterial infection since mice administered with rhDNase had reduced concentration of free DNA. Unfortunately, it did not alleviate the symptoms. The same study showed that NETs was implicated in systemic tissue damage in severe sepsis [37]. The results were consistent in cystic fibrosis patients whose most effective currently available therapy is rhDNase administration. rhDNase breaks down neutrophil DNA from mucus as

the rhDNase can much more quickly digest free DNA than DNA-protein complexes in NET structure [18, 38]. Although the role of NE is observed in the process, its function seems to be ambiguous. On the one hand, NE facilitates sputum dilution through histone degradation and provides access to rhDNases. On the other hand, NE release, similar to other proteolytic proteins, can damage lung tissue and enhance immune response through modulation of proinflammatory factors [18, 38].

It is worth mentioning, that NET may also be understood as a double-edge sword of innate immunity. Some bacteria were demonstrated to use their defense mechanisms that enabled them to avoid getting caught and killed by NETs [39]. One of the mechanisms is molecular mimicry whose objective is to hide molecules of antigenic bacteria to avoid immune response. A bacterial capsule is formed which contains a layer of polysaccharides similar to sialic acid of the host. The bacteria can also modify their surface through changing their charge from cation into anion. This is due to activation of *dlt* operon in bacterial genome which results in the rest of d-alanine getting bound to LTAs. Consequently, the charge is changed from negative to positive. The mechanism makes it possible to avoid immune response. Positively charged bacterial capsules repel NETs with AMPs [39]. In another defense mechanism used by pathogens to avoid getting caught in NETs, DNase is released. As it can break down DNA, the fundamental block of the network, it causes its degradation and dysfunction [35]. Although DNases are commonly released by Gram-negative bacteria, some cases of Gram-positive bacteria releasing DNases into extracellular environment were also recorded, including GAS – group A *Streptococcus* and *Streptococcus pneumoniae* [40]. *Streptococcus pneumoniae* are caught by the network of NETs and the *endA* gene encoding endonuclease A is expressed [6]. The presence of *endA* enzyme was shown to disturb the integrity of neutrophil granulocyte through its stimulation towards elastase release. Interestingly, *endA* release from *Streptococcus pneumoniae* in response to NET binding can be a factor of virulence of the bacteria. It is thanks to the enzyme that bacteria can spread further into the lungs and the circulatory system [40].

A study conducted on group A streptococcus showed that the group uses another enzyme, DNase Sda1, to escape NETs. It improves bacterial survival and their virulence both *in vivo* and *in vitro* [41]. Another bacterial defense mechanism is to trigger an exaggerated immune response as bacteria come into frequent contact with neutrophils, thus enhancing their proinflammatory activity. Group A strep (GAS) bacteria [42] were demonstrated to produce M1 protein, a key component for their virulence. M1 causes NET formation and release while at the same time

allows proteins from the network to escape degradation. Escape from neutrophil extracellular traps may also involve blocking the network from forming in neutrophils in the first place [17]. In *Streptococcus pyogenes* bacteria that produce SpyCEP protease, IL-8 is inactivated and GAS-stimulated NET formation rate is reduced which helps pathogens avoid extracellular killing [17].

#### NETs in viral infections

NETs can bind and immobilize viruses through electrostatic interactions between virus molecules and the chromatin skeleton of the network [43–46]. Additionally, histones present in NETs, rich in positively charged amino acids, can bind virus capsids negatively charged [43]. H3 and H4 histones were shown to aggregate in their structure type A influenza virus while H1 binds some noroviruses [43]. Recent research showed that some NET components, including myeloperoxidase, cathelicidin and  $\alpha$ -defensins, display strong antiviral properties. In particular,  $\alpha$ -defensins show their biocidal activity both in enveloped and non-enveloped viruses [43]. The human immunodeficiency virus HIV-1 was found to induce suicidal NETosis recognized by neutrophils mainly through TLR7 and TLR8 (Toll-like Receptors) [46]. The engagement of the receptors causes a cascade of events resulting in the production of ROS, followed by NET formation and destruction of the virus [46]. Interestingly, HIV-1 has developed defense mechanisms against NET. HIV-1 increases IL-10 production by DCs, holding against ROS generation and thus inhibiting NET release [46]. Influenza type A virus is another virus that can induce NETosis. The virus is associated with ARDS (acute respiratory distress syndrome) which causes NET formation following activation of epithelial cells of the lungs [47]. Other inducers of NETosis include oxidoreductases active in infection, super-oxidized ions and  $H_2O_2$ , from epithelial cells [43]. Hantaan virus also generates NET release and the cytotoxic properties of the network may exacerbate pathological changes of the organism induced by the virus infection [48]. NET histones generated in Hantaan virus infection facilitate thrombin generation and intravascular coagulation. They increase the permeability of vascular endothelial cells and induce production of antibodies thus exacerbating the pathological condition caused by the virus [48]. NS1 (non-structural protein 1) is the key protein that activates NETosis in Dengue virus infection [49]. The protein activates cells that have not been infected, including endothelial cells, via TLR4. As a result, endothelial cells stimulate neutrophils to form NETs. IL-8 produced by activated endothelial cells facilitates NET.

### NETs in fungal infections

NETs can inhibit the growth and development of *Candida albicans*, *Aspergillus nidulans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Candida glabrata* and *Cryptococcus gattii* [50, 51] although the mechanism behind this activity has not been fully explained. Calprotectin [50] was found to be the crucial protein involved in NET degradation of fungi. The fungicidal activity of calprotectin was found in a study on *Candida albicans*. Chelation of  $Zn^{2+}$  or  $Mn^{2+}$  ions, which are of importance for fungi, reduced their growth [50]. The same study showed that increased concentrations of the ions inhibit NET activity [50]. Furthermore, *in vitro* observations showed that when calprotectin is exhausted, NET inhibitory activity comes to an end [50]. Mice with calprotectin deficit are not able to prevent *C. albicans* infection [50]. It was also discussed that neutrophils sense microbe size, and that NET may be the mechanism of response to *Candida albicans* hyphae, but not to for yeast form [33]. Intriguingly, Branzk et al. [33] claim that NET is not forming in response to small yeast or single bacteria, whereas in different studies [52], it is claimed that hyphae, as well as yeast form can be caught by NET. The hyphae of *Candida albicans* after coming into contact with activated neutrophils cause histone degradation which affects further chromatin decondensation during NET release [33]. The study by Branzk et al. also shows [33] that selective death of fungus cells in the process of NETosis does not depend on enzymatic activity of the organisms as high temperature-inactivated *C. albicans* strands initiate NET release to the same extend as alive strands.

Similarly, in *Aspergillus fumigatus* infection, NETs are released in response to fungus strands or large agglomerates of conidia. However, NETs are not released when only individual conidia exist in extracellular environment [33]. Bruns et al. [52] demonstrated that strands of *A. fumigatus* are stronger inducers of NET formation than conidia. This finding can be linked to the presence of RodA hydrophobin. The protein occurs only on conidium surface which makes it immunologically indifferent and thus inhibits NET formation. This finding is consistent with another study [38] that showed that some components of fungal cell walls can trigger NET formation. The study demonstrated that while glucans initiate NET release in a ROS-regulated pathway mannans rely on a ROS-independent route [38].

### NETs in parasitic infections

NETs were identified in infections with *Leishmania*, *Plasmodium*, *Eimeria*, *Toxoplasma* and *Strongyloides* parasites [53–57]. Contact with these protozoa is possible only through a vector, e.g. a mosquito or a tick. A pathogen is inoculated into blood through its organism

[33]. NETs may be necessary to control parasites and modulate immune response through their constituent components [54]. Both promastigote and amastigote forms of parasites from the genus *Leishmania* (*L.*) are able to initiate NET formation, as was demonstrated by the presence of DNA strands in extracellular environment [54]. LPG found on the surface of promastigote cell form was shown to induce neutrophils into NET release. This is due to the fact that the negative charge on promastigote surface that regulates its binding to NETs can at least in part be also regulated by LPG presence [54]. The promastigote form of *L. donovani* is capable of NET induction although *L. donovani* and *L. major* promastigotes are resistant to NET-regulated killing [54].

NETs and histones present in the network are toxic for promastigotes [54]. A study conducted to identify susceptibility of successive species of *Leishmania* (*L.*) *sp.* to histone activity showed that promastigote forms of *L. mexicana*, *L. brasiliensis*, *L. major* and *L. amazonensis* are susceptible. Their protein-induced death mechanism remains to be explained. The amastigote forms of *L. mexicana* and *L. amazonensis* are resistant to the toxic activity of histones. Infection with *Plasmodium*, another protozoa, can stimulate NET formation. It was reported that NET is present in the blood of patients infected with *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium malariae* [56].

A role played by NETs was observed also in infections with *Eimeria bovis* [55]. The parasite was demonstrated to induce NET formation via calf neutrophils and to trap and bind the sporozoites. Infections with *Toxoplasma gondii* in humans and mice stimulates NET formation [47]. Bonne-Année et al. [53] demonstrated that NETs are also induced by *Strongyloides stercoralis*, a parasitic roundworm common in humans, primates and dogs. The study showed that although the network of neutrophil extracellular traps binds the parasites *in vitro*, it does not kill them [53].

### NETs in autoimmune diseases

NET dysfunction can result in infection or development of many non-infectious diseases in humans and in other mammals, including mice and cattle [58]. The accumulation of NETs released during infection or pathological condition can injure adjacent tissue. Irregular removal of NETs from the organism causes pathological conditions in the macroorganism. Endogenous DNase may be implicated in the process since their reduced levels disturb successful NET degradation [59]. On the other hand, destruction of DNA skeleton, a NET element, releases antibacterial proteins which can cause massive damage of adjacent tissues [59]. For example, myeloperoxidase released in this way inactivates metalloprotease inhibitors. Consequently, pathological changes caused by

the enzymes can occur. Another example is provided by neutrophil elastase release from NET skeleton which can damage barriers formed by the epithelium, e.g. in the lungs [59].

One of the negative effects of NET presence in extracellular environment is that NETs are involved in autoimmune diseases. During NET formation, citrullinated histones and antigens are released into extracellular environment and are presented to immunocompetent cells which in response start producing ANCAs and ANAs [60, 61]. The antibodies usually act against myeloperoxidase, proteinase 3 and neutrophil elastase. As a result, neutrophils get activated and NETs are released [15]. NETs contain autoantigens, including myeloperoxidase and proteinase 3, which become the target of successive antibodies. Thus, an autoactivation loop is created which is the underlying component of autoimmune disease development. Abnormal NET formation, issues with network degradation and lack of equilibrium between the two are the factors initiating or facilitating autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis and vasculitis [59–63]. Antibodies are produced by B lymphocytes against nuclear antigens, e.g. DNA, histones or RNPs and against ANCAs, in SLE [59]. That leads to a typical feature of the disease, i.e. formation of immune complexes and long-term activation of pDCs [59]. Abnormal removal of cells undergoing apoptosis was recorded in which the release of intracellular autoantigens caused constant production of autoantibodies which is considered to be the key mechanism of the pathogenesis of the disease [59]. The mechanism of its pathophysiology has a role for genes, hormones, environment and abnormal lymphocytes which may affect development of the disease [59]. Having described cell death in NETosis, NETs became the successive and likely sources of autoantigens produced in the disease. Thus, the phenomenon was redefined as one cause of the disease [59]. A link between autoantigen-releasing cells, including plasmacytoid dendritic cells, B lymphocytes, antibodies, and NETs was demonstrated [64]. Neutrophils primed with type I interferons and exposed to autoantibodies produced NETs [51]. NET components can then activate B lymphocytes via their specific BCRs and TLR-9. Then follows production of autoantibodies against their own DNA and cathelicidin (LL-37) which occur in large amounts in SLE patients [65]. The antibodies can bind DNA and cathelicidin (LL-37) from NETs and form autoantibody-DNA/LL-37 complexes which are recognized by B cells and pDCs and then “sustain” autoimmune infections [65]. A correlation between insufficient NET degradation and development of the disease was found in SLE [59]. The serum of SLE patients had reduced ability to degrade NETs. Accordingly, patients could be divided into non-degraders and degraders with higher NET degradation activity [59].

Another study on SLE [61] showed that the ability to degrade NETs is correlated with its activity. Accordingly, observations showed that increased ability to NET degradation occurred in remission and decreased ability to do so happened in relapses. Moreover, patients with worsened condition had increased levels of NET antibodies. Undegraded NET in SLE was demonstrated to activate the complement system and C1q, a component of the system degrades NETs. The opsonization of NETs or direct inhibition of DNase I are the hypothesized origins of the phenomenon [61].

Another autoimmune disease that involves NETs is rheumatoid arthritis (RA). The serum of RA patients contains a range of autoantibodies, including antifilagrin antibodies, anti-creatinine antibodies and antiperinuclear factors [4, 63]. The serum can be used to obtain fundamental diagnostic parameters, including rheumatoid factor, ACPAs and importantly anti-citrullinated protein antibodies [63]. Proteins that citrullinate during NET formation have increased antigenicity which generates production of their antibodies [53]. Khandpur et al. showed [63] that there is a correlation between NETs and ACPA presence and concentration in the blood. RA serum antibodies were observed to react with NET histones [63]. Therefore, NETs are speculated to display immune properties which can trigger a vicious circle of immune reactions. ACPA-binding NETs in the joint lead to the neutrophil Fcγ receptor binding to immune complex which further facilitates NET release [63]. A correlation was found between NET formation and systemic inflammatory markers [63]. Khandpur et al. [63] showed that neutrophils taken from RA patients are more prone to form NETs than those from healthy individuals. IL-17 and TNF-α also derived from the serum are able to induce NETs in RA neutrophils.

#### NETs in cancer

Anticancer effect of neutrophils is known for several years [66]. Following direct interactions, neutrophils induce migration of cancer cells, simulate their growth via metalloproteinases released from the extracellular matrix and facilitate angiogenesis [66]. Conversely, activated neutrophils exert cytotoxic effect on cancer cells by the release of ROS and defensins [66]. G-CSF that is produced by most types of cancer cells activates neutrophils and stimulates them to NET formation [66]. Some NET components, including myeloperoxidase, proteinases and histones, can have a cytotoxic impact on cancer cells and inhibit cancer growth [66]. NETs are suggested to bind cancer cells, thus blocking their further proliferation in the organism [66].

The alternative view on the role of NETs says that through the activation of its protein components the network can facilitate extravasation and metastasis [67].

NETs are thought to ultimately stick to metastasis cells. Through the recruitment of platelets, NETs can protect cancer cells and inhibit immune response [67]. NETs were also shown to directly trigger malignant transformation of cancer [67]. In a study conducted on cancer mice, infection-induced NETs could facilitate metastasis through trapping cancer cells in the circulatory system and facilitate tumor relapse after its surgical resection in patients with metastatic colorectal cancer [68].

Recent studies of Albregues et al. [69], it was reported that sustained lung inflammation induced the NET formation and awaken dormant cancer. It was shown [1], that two components of NET – NE and MMP9 caused laminin cleavage, which induced proliferation of dormant cancer cells. Moreover, antibodies against NET-remodeled laminin prevented awaking of dormant cells, giving the first step of a new pathway to prolong the survival of cancer patients [69].

#### NETs in pregnancy and fertility

The negative effect of NETs was observed in human fetal development, e.g. in preeclampsia [70, 71]. In an *in vitro* study on CD11b expression, fragments of placenta were able to induce neutrophils into the formation of NETs [70, 71]. Contrary to healthy placenta, the placenta of preeclampsia women had NETs occurring in close contact with trophoblasts [70, 71]. NETs are thus hypothesized to take the space inside trophoblast villi, to reduce vascular blood flow and to finally cause fetal hypoxia [70]. Another mechanism that can result in NET-assisted fetal hypoxia is NETosis facilitated by activated vascular endothelial cells [70]. which eventually destroys endothelial cells of the mother [70, 71]. It is unknown at this point whether NETs trigger pathological changes or the network is caused by some type of deficiency in the placenta [70, 71]. Analysis of levels of free DNA circulating in the mother and the fetus in preeclampsia showed that NETs must have been its origin. The growth of cfDNA (cell-free DNA) inside the fetus can suggest a pathological condition of the placenta [70, 71]. However, a high level of maternal free DNA suggest that its origin lies in NET generation [70, 71]. Research showed that increase of fetal cfDNA levels occurs in early pregnancy, prior to preeclampsia symptoms. However, increased maternal free DNA occurs only once the symptoms develop [64]. Since ROSs are necessary for NET formation and NET release into extracellular environment, some researchers speculate that NETs can contribute to miscarriages caused by maternal antiphospholipid antibodies [71]. Hahn et al. [71] contend that the presence of NETs can be cytotoxic for adjacent tissues, which can have an adverse effect on the fetus.

NETs in animals, mainly mares, can constitute a distinctive feature of individuals with reduced fertility [72].

The reason behind it was thought to be the ability of neutrophils to bind the spermatozoo and form widespread agglomerates which reduces sperm motility and inhibits its ability to fertilize the egg. Following NET discovery, the ability of sperm to form sperm agglomerates using NETs was investigated [72]. Spermatozoids were shown to activate neutrophils to NET formation and release in a way similar to that in which bacteria activate cells [72]. Since SP (seminal plasma) contains proteins with endonuclease capabilities, similar to DNases - e.g. DNase I, it increases sperm fertility potential in cattle, horses, and pigs through their release from NET agglomerates [72].

#### Conclusions

NET formation has focused the attention of many scientists. It seems to be not only a very effective defense mechanism used by immune system cells (neutrophils) but also a structure that inevitably requires a “victim” i.e. NETosis, or cell death. NETs can act like a double-edged sword. The positive effects of NETs in pathogenic infections (bacteria, viruses, fungi and parasites) are balanced by their negative effect on the health of mammals, including humans. NETs are observed in non-infectious, including autoimmune diseases, cancer, fertility disorders in humans and animals. More recent reports implicate the role of NET formation in Alzheimer’s disease and sterile inflammation.

#### Abbreviations

ACPA: Anti-citrullinated protein antibodies; AMP: Antimicrobial peptides; ANAs: Anti-nuclear antibodies; ANCAs: Anti-neutrophil cytoplasmic antibodies; BET: Basophil extracellular trap; EET: Eosinophil extracellular trap; G-CSF: Granulocyte-colony stimulating factor; HET: Heterophil extracellular trap; LPG: Lipophosphoglycan; LPS: Lipopolysaccharide; LTA: Lipoteichoic acid; MCET: Mast cell extracellular trap; MET: Macrophage/monocyte extracellular trap; MMP9: Metalloproteinase 9; MPO: Myeloperoxidase; NE: Neutrophil elastase; NET: Neutrophil extracellular trap; PAF: Platelet-activating factor; PMA: Phorbol myristate acetate; PMN: Polymorphonuclear leukocytes; RA: Rheumatoid arthritis; RNPs: Ribonucleoproteins; ROS: Reactive oxygen species; SLE: Systemic lupus erythematosus; TNF $\alpha$ : Tumor necrosis factor

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**References**

- Amulic B, Hayes G. Neutrophil extracellular trap. *Curr. Biol.* 2011;21:297–8.
- Kolaczowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat. Rev. Immunol.* 2013;13:159–75.
- Medina E. Neutrophil extracellular traps: A strategic tactic to defeat pathogens with potential consequences for the host. *J. Innate Immunol.* 2009;1:176–80.
- Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. *Nat. Immunol. Rev.* 2017;18:134–47.
- Vorobjeva NV, Pinegin BV. Neutrophil extracellular traps: mechanisms of formation and role in health and disease. *Biochemistry (Mosc).* 2014;79:1286–96.
- Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. *Science.* 2004;303:1532–5.
- Goldmann O, Medina E. The expanding world of extracellular traps: not only neutrophils but much more. *Front. Immunol.* 2013;3. <https://doi.org/10.3389/fimmu.2012.00420>.
- Schauer C, Janko C, Munoz LE, Zhai Y, Kienhöfer D, Frey B, Lell M, Manger B, Rech J, Naschberger E, Holmdahl R, Krenn V, Harrer T, Jeremic I, Bilyy R, Schett G, Hoffmann M, Herrmann M. Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. *Nat. Med.* 2014;20:511–7.
- Dworski R, Simon H-U, Hoskins A, Yousefi S. Eosinophil and neutrophil extracellular DNA traps in human allergic asthmatic airways. *J. Allergy Clin. Immunol.* 2011;127:1260–6.
- Aulik NA, Hellenbrand KM, Czuprynski CJ. *Mannheimia haemolytica* and its leukotoxin cause macrophage extracellular trap formation by bovine macrophages. *Infect. Immun.* 2012;80:1923–33.
- Campillo-Navarro M, Leyva-Paredes K, Donis-Maturano L, Gonzalez-Jimenez M, Paredes-Vivas Y, Cerbulo-Vazquez A, Serafin-Lopez J, Garcia-Perez B, Ullrich SE, Flores-Romo L, Perez-Tapia S, Estrada-Parra S, Estrada-Garcia I, Chacon-Salinas R. *Listeria monocytogenes* induces mast cell extracellular traps. *Immunobiology.* 2017;222:432–9.
- Von Köckritz-Blickwede M, Goldmann O, Thulin P, Heinemann K, Norrby-Teglund A, Rohde M, Medina E. Phagocytosis-independent antimicrobial activity of mast cells by means of extracellular trap formation. *Blood.* 2008;111:3070–80.
- Yousefi S, Morshed M, Amini P, Stojkov D, Simon D, von Gunten S, Kaufmann T, Simon H-U. Basophil exhibit antibacterial activity through extracellular trap formation. *Allergy.* 2015;70:1184–8.
- Chuammitri P, Ostojic J, Andreasen CB, Redmond SB, Lamont SJ, Palic D. Chicken heterophil extracellular traps (HETs): novel defense mechanism of chicken heterophils. *Vet. Immunol. Immunopath.* 2009;129:126–31.
- Palic D, Ostojic J, Andreasen JB, Roth JA. Fish cast NETs: neutrophil extracellular traps are released from fish neutrophils. *Dev. Comp. Immunol.* 2007;31:805–16.
- Soderhaall K, Smith VJ. Separation of the haemocyte populations of *Carcinus maenas* and other marine decapods, and prophenoeloxidase distribution. *Dev. Comp. Immunol.* 1983;7:229–39.
- Carestia A, Kaufman T, Schattner M. Platelets: New brick in the building of neutrophil extracellular traps. *Front. Immunol.* 2016;7. <https://doi.org/10.3389/fimmu.2016.00271>.
- Von Köckritz-Blickwede M, Nizet V. Innate immunity turned inside-out: antimicrobial defense by phagocyte extracellular traps. *J. Mol. Med.* 2009;87:775–83.
- Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MK, Patel KD, Chakrabarti S, McAvoy E, Sinclair GD, Keys EM, Allen-Vercoe E, DeVinney R, Doig CJ, Green FHY, Kubes P. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat. Med.* 2007;13:463–9.
- Röhm M, Grimm MJ, D'Auria AC, Almyroudis NG, Segal BH, Urban CF. NADPH oxidase promotes neutrophil extracellular trap formation in pulmonary aspergillosis. *Infect. Immun.* 2014;82:1766–77.
- Nishinaka Y, Arai T, Adachi S, Takaori-Kondo A, Yamashita K. Singlet oxygen is essential for neutrophil extracellular trap formation. *Biochem. Biophys. Res. Commun.* 2011;413:75–9.
- Miyamoto S, Martinez GR, Rettori D, Augusto O, Medeiros MHG, di Mascio P. Linoleic acid hydroperoxide reacts with hypochlorous acid, generating peroxy radical intermediates and singlet molecular oxygen. *Proc. Natl. Acad. Sci. USA.* 2006;103:293–8.
- Douda DN, Khan MA, Grasmann H, Palaniyar N. SK3 channel and mitochondrial ROS mediate NADPH oxidase-independent NETosis induced by calcium influx. *Proc. Natl. Acad. Sci. USA.* 2015;112:2817–22.
- Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. *J. Exp. Med.* 2010;207:1853–62.
- Itakura A, McCarty OJ. Pivotal role for the mTOR pathway in the formation of neutrophil extracellular traps via regulation of autophagy. *Am. J. Physiol. Cell Physiol.* 2013;305:C348–54.
- Majewski P, Majchrzak-Gorecka M, Grygier B, Skrzeczynska-Moncznik J, Osiecka O, Cichy J. Inhibitors of Serine Proteases in Regulating the Production and Function of Neutrophil Extracellular Traps. *Front. Immunol.* 2016;7. <https://doi.org/10.3389/fimmu.2016.00261>.
- Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science.* 2015;349:316–20.
- Leppkes M, Maueröder C, Hirth S, Nowecki S, Günther C, Billmeier U, Paulus S, Biermann M, Munoz LE, Hoffmann M, Wildner D, Croxford AL, Waisman A, Mowen K, Jenne DE, Krenn V, Mayerle J, Lerch MM, Schett G, Wirtz S, Neurath MF, Herrmann M, Becker C. Externalized decondensed neutrophil chromatin occludes pancreatic ducts and drives pancreatitis. *Nat. Commun.* 2016;7. <https://doi.org/10.1038/ncomms10973>.
- Papayannopoulos V, Metzler MD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J. Cell Biol.* 2010;191:677–91.
- Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, Patel KD, Chakrabarti S, McAvoy E, Sinclair GD, Keys EM, Allen-Vercoe E, DeVinney R, Doig CJ, Green FHY, Kubes P. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat. Med.* 2007;13:463–469.
- Pilszczek FH, Salina D, Poon KK, Fahey C, Yipp BG, Sibley CD, Robbins SM, Green FH, Surette MG, Sugai M, Bowden MG, Hussain M, Zhang K, Kubes P. A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to *Staphylococcus aureus*. *J. Immunol.* 2010;185:7413–25.
- Byrd AS, O'Brien XM, Johnson CM, Lavigne LM, Reichner JS. An extracellular matrix-based mechanism of rapid neutrophil extracellular trap formation in response to *Candida albicans*. *J. Immunol.* 2013;190:4136–48.
- Branzk N, Lubojemska A, Hardison SE, Wang Q, Gutierrez MG, Brown GD, Papayannopoulos V. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. *Nat. Immunol.* 2014;15:1017–25.
- Kumar V, Sharma A. Neutrophils: Cinderella of innate immune system. *Int. Immunopharmacol.* 2010;10:1325–34.
- Halverson TW, Wilton M, Poon KK, Petri B, Lewenza S. DNA is an antimicrobial component of neutrophil extracellular traps. *PLoS Pathol.* 2015;11. <https://doi.org/10.1371/journal.ppat.1004593>.
- Bystrzycka W, Moskalik A, Siczowska S, Manda-Handzlik A, Demkow U, Ciepiela O. The effect of clindamycin and amoxicillin on neutrophil extracellular trap (NET) release. *Centr. Eur. J. Immunol.* 2016;41:1–5.
- Czaikoski PG, Mota JM, Nascimento DC, Sonego F, Castanheira FV, Melo PH, Scorteganga GT, Silva RL, Barroso-Sousa R, Souto FO, Pazin-Filho A, Figueiredo F, Alves-Filho JC, Cunha FQ. Neutrophil extracellular traps induce organ damage during experimental and clinical sepsis. *PLoS One.* 2016;5. <https://doi.org/10.1371/journal.pone.0148142>.
- Zawrotniak M, Bochenska O, Karkowska-Kuleta J, Seweryn-Ozog K, Aoki W, Ueda M, Andrzej KA, Rapala-Kozik M. Aspartic proteases and major cell wall components in *Candida albicans* trigger the release of neutrophil extracellular traps. *Front. Cell Infect. Microbiol.* 2017;7. <https://doi.org/10.3389/fcimb.2017.00414>.
- Wartha F, Beiter K, Albiger B, Fernebro J, Zychlinsky A, Normark S, Henriques-Normark B. Capsule and D-alanylated lipoteichoic acids protect

- Streptococcus pneumoniae* against neutrophil extracellular trap. *Cell Microbiol.* 2007;9:1162–71.
40. Buchanan JT, Simpson AJ, Aziz RK, Kristian SA, Kotb M, Feramisco J, Nizet V. DNase expression allows the pathogen group A *Streptococcus* to escape killing in neutrophil extracellular traps. *Curr. Biol.* 2006;16:396–400.
  41. Beiter K, Wartha F, Albiger B, Normark S, Zychlinsky A, Henriques-Normark B. An endonuclease allows *Streptococcus pneumoniae* to escape from neutrophil extracellular traps. *Curr. Biol.* 2006;21:401–7.
  42. Lauth X, von Köckritz-Blickweide M, McNamara CW, Myskowski S, Zinkernagel AS, Beall B, Ghosh P, Gallo RL, Nizet V. M1 protein allows group A streptococcal survival in phagocyte extracellular traps through cathelicidin inhibition. *J. Innate Immunol.* 2009;1:202–14.
  43. Schönrich G, Raftery MJ. Neutrophil extracellular trap go viral. *Front Immunol.* 2016;7. <https://doi.org/10.3389/fimmu.2016.00366>.
  44. Wardini AB, Guimaraes-Costa AB, Nascimento MT, Nadaes NR, Danelli MG, Mazur C, Benjamin CF, Saraiva EM, Pinto-da-Silva LH. Characterization of neutrophil extracellular traps in cats naturally infected with feline leukemia virus. *J. Gen. Virol.* 2010;91:259–64.
  45. 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.
  46. Saitoh T, Komano J, Saitoh Y, Misawa T, Takahama M, Kozaki T, Uehata T, Iwasaki H, Omori H, Yamaoka S, Yamamoto N, Akira S. Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. *Cell Host Microbe.* 2012;12:109–16.
  47. Narayana MA, Narasaraju T, Rai P, Perumalsamy R, Tan KB, Wang S, Engelward B, Chow VT. In vivo and in vitro studies on the roles of neutrophil extracellular traps during secondary pneumococcal pneumonia after primary pulmonary influenza infection. *Front Immunol.* 2013;4:56.
  48. Kobak L, Raftery MJ, Voigt S, Kuhl AA, Kilic E, Kurth A, Witkowski P, Hofmann J, Nitsche A, Schaade L, Krüger DH, Schönrich G. Hantavirus-induced pathogenesis in mice with a humanized immune system. *J. Gen. Virol.* 2015;96:1258–63.
  49. Modhiran N, Watterson D, Muller DA, Panetta AK, Sester DP, Liu L, Hume DA, Stacey KJ, Young PR. Dengue virus NS1 protein activates cells via toll-like receptor 4 and disrupts endothelial cell monolayer integrity. *Sci. Transl. Med.* 2015;304:304ra142.
  50. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, Brinkmann V, Jungblut PR, Zychlinsky A. Neutrophils extracellular traps contain calprotectin, a cytosolic protein complex involved in host defence against *Candida albicans*. *Plos Pathog.* 2009;5. <https://doi.org/10.1371/journal.ppat.1000639>.
  51. Urban CF, Reichard U, Brinkmann V, Zychlinsky A. Neutrophil extracellular traps capture and kill *Candida albicans* yeast and hyphal forms. *Cell Microbiol.* 2006;8:668–76.
  52. Bruns S, Kniemeyer O, Hasenberg M, Amanianda V, Nietzsche S, Thywissen A, Jeron A, Latgé JP, Brakhage AA, Gunzer M. Production of extracellular traps against *Aspergillus fumigatus* in vitro and by infected lung tissue is dependent on invading neutrophils and influenced by hydrophobin RodA. *PLoS Pathog.* 2010;6. <https://doi.org/10.1371/journal.ppat.1000873>.
  53. Bonne-Année S, Kerepesi LA, Hess JA, Wesolowski J, Paumet F, Lok JB, Nolan TJ, Abraham D. Extracellular traps are associated with human and mouse neutrophil and macrophage mediated killing of larval *Strongyloides stercoralis*. *Microbes Infect.* 2014;16:502–11.
  54. Guimarães-Costa AB, Nascimento MTC, Forment GS, Soares RPP, Morgado FN, Conceição-Silva F, Saraiva E. *Leishmania amazonensis* promastigotes induce and are killed by neutrophil extracellular traps. *Proc. Natl. Acad. Sci. USA.* 2009;106:748–53.
  55. Behrendt JH, Ruiz A, Zahner H, Taubert A, Hermsilla C. Neutrophil extracellular trap formation as innate immune reactions against the apicomplexan parasite *Eimeria bovis*. *Vet Immunol. Immunopathol.* 2010;133:1–8.
  56. Kho S, Minigo G, Andres B, Leonardo L, Prayoga P, Poespoprodjo JR, Kenangalem E, Proce RN, Woodberry T, Anstey NM, Yeo TW. Circulating neutrophil extracellular traps and neutrophil activation are increased in proportion to disease severity in human malaria. *J. Infect. Dis.* 2019;219:1994–2004.
  57. Abi Abdallah DS, Lin C, Ball CJ, King MR, Duhamel GE, Denkers EY. *Toxoplasma gondii* triggers release of human and mouse neutrophil extracellular traps. *Infect. Immun.* 2012;80:768–77.
  58. Remijsen Q, Kuijpers TW, Wirawan E, Lippens S, Vandenabeele P, Vanden BT. Dying for a cause: NETosis, mechanisms behind an microbial cell death modality. *Cell Death Differ.* 2011;18:581–8.
  59. Hakkim A, Fürnrohr BG, Amann K, Laube B, Abed UA, Brinkmann V, Herrmann M, Voll RE, Zychlinsky A. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proc. Natl. Acad. Sci. USA.* 2010;107:9813–8.
  60. Panda R, Krieger T, Hopf L, Renne T, Haag F, Rober N, Conrad K, Csernok E, Fuchs TA. Neutrophil extracellular traps contain selected antigens of anti-neutrophil cytoplasmic antibodies. *Front Immunol.* 2017;8. <https://doi.org/10.3389/fimmu.2017.00439>.
  61. Loeffler J, Martin M, Gullstrand B, Tydén H, Lood C, Truedsson L, Bengtsson AA, Blom AM. Neutrophil extracellular traps that are not degraded in systemic lupus erythematosus activate complement exacerbating the disease. *J. Immunol.* 2012;188:3522–31.
  62. Lood C, Blanco LP, Purmalek MM, Carmona-Rivera C, De Ravin SS, Smith CK, Knight JS, Friday S, Li S, Patel RM, Subramanian V, Thompson P, Chen P, Fox DA, Pennathur S, Kaplan MJ. NET are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Sci. Transl. Med.* 2013;5. <https://doi.org/10.1126/scitranslmed.3005580>.
  64. Lande R, Gregorio J, Facchinetti V, Chattejee B, Wang YH, Homey B, Cao W, Wang YH, Su B, Nestle FO, Zal T, Mellman I, Schröder JM, Liu YJ, Gilliet M. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature.* 2007;449:564–9.
  65. Garcia-Romo GS, Caielli S, Vega B, Connolly J, Allantaz F, Xu Z, Punaro M, Baisch J, Guiducci C, Coffman RL, Barrat FJ, Banchemareau J, Pascual V. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci. Transl. Med.* 2011;3:73ra20.
  66. Souto JC, Vila L, Brú A. Polymorphonuclear neutrophils and cancer: intense and sustained neutrophilia as a treatment against solid tumors. *Med. Res. Rev.* 2011;31:311–63.
  67. Olsson A-K, Cedervall J. NETosis in cancer – platelet-neutrophil crosstalk promotes tumor-associated pathology. *Front Immunol.* 2016;7. <https://doi.org/10.3389/fimmu.2016.00373>.
  68. Tohme S, Yazdani HO, Al-Khafaji AB, Chidi AP, Loughran P, Mowen K, Wang Y, Simmons RL, Huang H, Tsung A. Neutrophil extracellular traps promote the development and progression of liver metastases after surgical stress. *Cancer Res.* 2016;76:1367–80.
  69. Albrengues J, Shields MA, Ng D, Park CG, Ambrico A, Poindexter ME, Upadhyay P, Uyeminami DL, Pommier A, Küttner V, Bružas E, Maiorino L, Bautista C, Carmona EM, Gimotty PA, Fearon DT, Chang K, Lyons SK, Pinkerton KE, Trotman LC, Goldberg MS, Yeh JT-H, Egeblad M. Neutrophils extracellular traps produced during inflammation awaken dormant cancer cells in mice. *Science.* 2018;361:eaao4227.
  70. Brinkmann V, Zychlinsky A. Neutrophil extracellular traps: Is immune the second function of chromatin? *J. Cell Biol.* 2012;198:773–83.
  71. Hahn S, Giaglis S, Hoesli I, Hasler P. Neutrophil NET in reproduction: from infertility to preeclampsia and the possibility of fetal loss. *Front Immunol.* 2012;3. <https://doi.org/10.3389/fimmu.2012.00362>.
  72. Alghamdi AS, Foster DN. Seminal DNase frees spermatozoa entangled in neutrophil extracellular traps. *Biol. Reprod.* 2005;73:1174–81.

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