

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# The Role of Neutrophil Extracellular Traps in Post-Injury Inflammation

---

Eszter Tuboly, Gabrielle D. Briggs and Zsolt J. Balogh

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.68906>

---

## Abstract

Polymorphonuclear (neutrophil) granulocytes (PMNs) are an essential part of the innate immune responses and key instigators and effectors of the underlying pathological mechanisms (endothelial damage, interstitial histolysis, cytokine production, phagocytosis) leading to post-injury inflammation and secondary tissue injury. In 2004, the formation of neutrophil extracellular traps (NETs) was identified as an additional defence mechanism of PMN against microbes. The understanding of complex regulation of neutrophil functions and NET formation is essential for differentiating between healthy and pathological inflammatory response, which frequently determines if patient recovers uneventfully or develops catastrophic complications. Recent discoveries have revealed the potential role of NETs in the pathogenesis of a wide range of non-infectious diseases, including post-injury sterile inflammation. In such conditions, both spontaneous NET formation and impaired NETosis are documented. In this chapter, we review the evidence for the role of NETs in post-injury inflammation, the key molecular and cellular participants in pathological NET formation, the clinical relevance of NETs in post-injury complications and the therapeutic potential of NET inhibition/clearance.

**Keywords:** neutrophil granulocyte, PMN, post-injury inflammation, neutrophil extracellular traps, trauma, injury, multiple organ failure

---

## 1. Introduction

Despite recent improvements in the care of the injured, severe trauma remains a major burden on our society, resulting in the annual death of more than five million people worldwide (World Health Organisation. Injuries and violence: the facts. 2014. <http://www.who>.

int/violence\_injury\_prevention/key\_facts/en/ Accessed 9 May 2016). Tissue injury, traumatic shock and subsequent resuscitation and surgical interventions lead to localised and systemic inflammatory responses. Polymorphonuclear (neutrophil) granulocytes (PMNs) are an essential part of the innate immune responses and key instigators and effectors of the underlying pathological mechanisms (endothelial damage, interstitial histolysis, cytokine production, phagocytosis) leading to post-injury inflammation and secondary tissue injury. In 2004, the formation of neutrophil extracellular traps (NETs) was identified as an additional defence mechanism of PMN against microbes [1]. Since the initial description of their antibacterial function, a series of studies reported the existence of NETs in response to various types of sterile inflammations including traumatic injury [2–5]. The precise triggers, contributions and outcomes of NETs in trauma patients are not well understood. Given the significant clinical impact of sterile inflammation in these patients, understanding the role of NETosis may identify novel biomarkers or therapeutic strategies to minimise post-injury tissue damage and hyperinflammation. In this chapter, we summarise our current knowledge and existing gaps on post-injury NET formation.

## 2. Post-injury inflammation

### 2.1. Complications of post-injury inflammation

Major trauma patients universally develop systemic inflammatory response syndrome (SIRS) criteria within 72 h of injury. SIRS is defined by the following criteria:

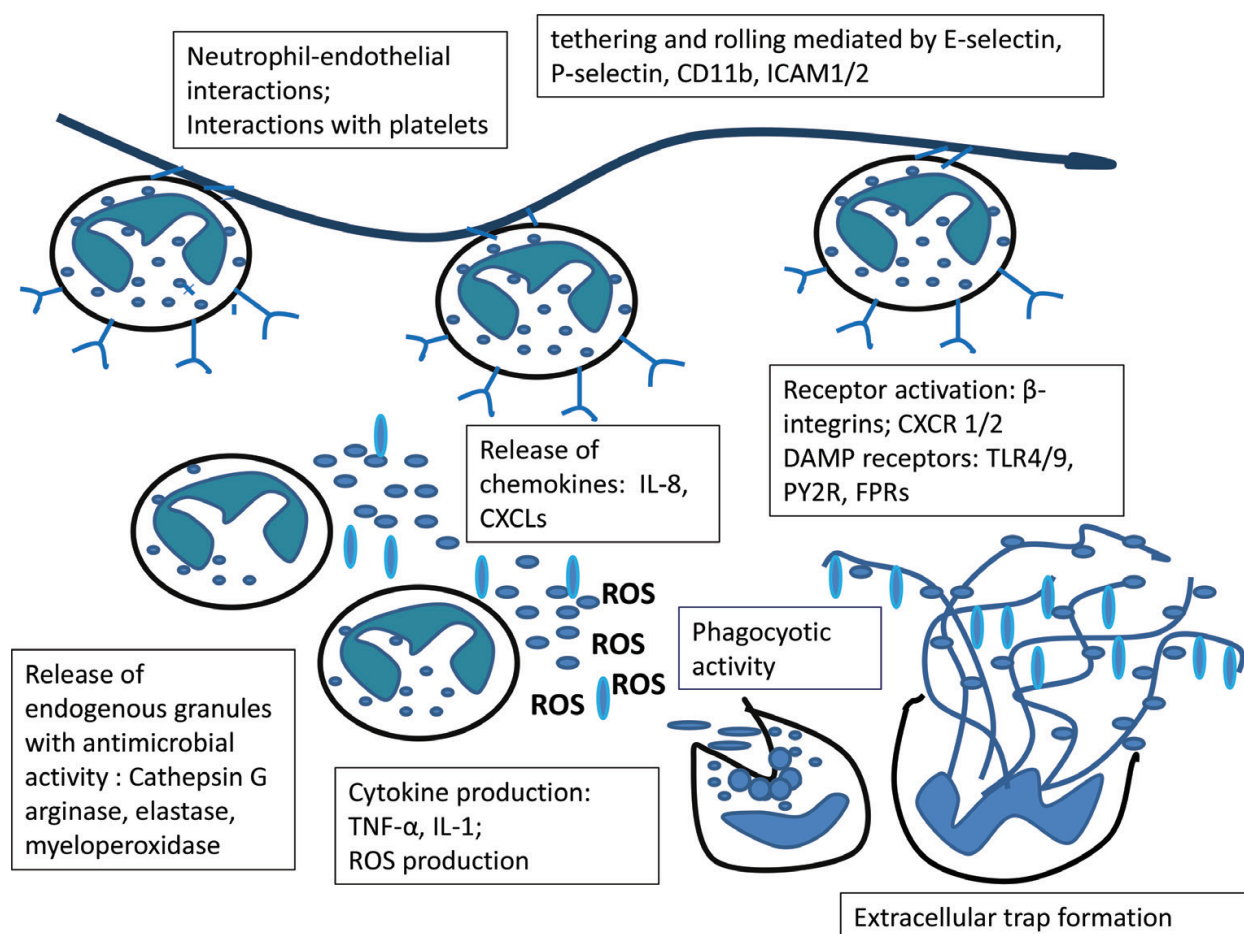
- a. Temperature greater than 38°C or less than 36°C.
- b. Heart rate greater than 90 beats/min.
- c. Respiratory rate greater than 20/min.
- d. White blood cell count (WBC) greater than  $12.0 \times 10^9 \text{ L}^{-1}$ , or less than  $4.0 \times 10^9 \text{ L}^{-1}$  [6].

The degree of the dysfunctional post-injury inflammation is further complicated by the invasive nature of surgical procedures. Moreover, those who survive the initial severe tissue injury and traumatic shock are at an increased risk of acute respiratory distress syndrome (ARDS), multiple organ failure (MOF), nosocomial infections and sepsis. These complications lead to excessive resource utilisation and increased risk of death [7–9]. The systemic inflammatory response to major trauma can lead to the development of early MOF, which progresses to a state of immune paralysis and is viewed as a major factor underlying the increased susceptibility of trauma patients to hospital-acquired infections [10, 11]. The possible involvement of NETs in post-injury inflammation has been evaluated in several recent studies. Margraf and co-workers published in 2008 that NET quantities in plasma may predict MOF and sepsis on the ICU in patients after multiple trauma [12], and more recently, cell free-DNA neutrophil extracellular traps (cf-DNA/NETs) were used in the prediction of mortality in a population of 32 patients with severe burn injury [13]. These associations warrant further research

into the precise role and impact of NETosis in the post-injury inflammatory response. While many aspects of the post-injury inflammatory response have been characterised over the past decades, our understanding of how NETosis fits into the picture is still rudimentary.

## 2.2. Mechanisms of post-injury inflammation

In the bigger picture of the post-injury inflammatory response, NETosis is considered a later phenomenon than the classical neutrophils functions [14–16]. Before the induction of NETosis, inflammatory reactions triggered by mechanical injury or disturbances of homeostasis are mainly propagated by intravascular events, summarised in **Figure 1**. The acute phase is characterised by dramatic changes in the diameter of the capillaries and the activation of innate immune cell responses. It is followed by a delayed, subacute reaction, most prominently characterised by oxido-reductive burst, hypoxic metabolic pathways, the infiltration of leukocytes and phagocytic cells and early cytokine production, while in the late proliferative phase, reperfusion injury, further production of late inflammatory agents, tissue remodelling and fibrosis occur.



**Figure 1.** Schematic figure about multiple functions of neutrophils in response to sterile inflammation, where CD11b, integrin  $\alpha$  M; ICAM, intercellular adhesion molecule; IL-8, interleukin-8 (chemokine receptor ligand 8); CXCLs, chemokine ligands; CXCR, chemokine receptor; DAMP, damage associated molecular pattern; TLR, Toll like receptor; IL-1, interleukin 1; PY2R, purinergic receptor; FPR, formyl peptide receptors; and  $\text{TNF}\alpha$ , tumor necrosis factor-alpha.

Injury leads to the release of damage-associated molecular patterns (DAMPs) with high immunomodulatory potential (extracellular DNA, mitochondrial remnants and the high mobility group box 1) and pro-inflammatory cytokines, such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), or interleukin-1 $\beta$  (IL-1 $\beta$ ). Release of these components results in Toll-like receptor (TLR) activation with an effect after 1–2 h [17]. As this phase ensues, subacute cytokines including IL-6, IL-8 as well as IL-12 and IL-18, chemokines and leukocyte migratory factors drive an exaggerated activation of PMN leukocytes, and the increased production of reactive oxygen species (ROS) plays important roles in the process [18]. It is also widely accepted that the initial pro-inflammatory phase switches to a later anti-inflammatory phase with extended anti-inflammatory cytokine release to facilitate regenerative processes; however, the pro-inflammatory and anti-inflammatory forces may ultimately reinforce each other, creating a state of increasingly destructive immunologic dissonance [19]. Cytokine signals are crucial in the inflammatory cascade by promoting the interactions of PMN leukocytes with endothelial cells through the up-regulation of adhesion molecules, PMN degranulation, respiratory burst, lipid mediator synthesis [20] and enhanced migration through the endothelium. Via these reactions, the soluble mediators alter the microvascular homeostasis [21, 22] and blood flow, which have been associated with multiple organ failure [23]. Of the cytokines, members of the low molecular weight chemokine family play a fundamental part in these events by virtue of their ability to attract and stimulate leukocytes [24]. These mediators mutually and strictly regulate the expression level and generation of each via epigenetic regulation that propagate the commencement of repair mechanisms, although numerous cytokines are reported to be aberrantly regulated in association with more complicated clinical outcomes [25, 26].

While phagocytosis and degranulation usually take minutes to occur after being exposed to the inflammatory signal, NETosis is a more protracted event, takes place from 2–3 h up to 8 h from activation [27, 28]. About 20–60% of isolated human neutrophils typically release NETs 2–4 h after stimulation with microbes or chemicals [2]. However, they were able to respond within minutes when activated by LPS-stimulated platelets under conditions of flow [29]. These studies suggest that NET formation might be more characteristic for the subacute/late phase of post-injury inflammation and probably more inherent to the senescent PMN population. It is hoped that future studies will identify which factors determine the selection between these alternative antimicrobial activities and whether these processes can coexist in the same cell (**Figure 1**).

### 3. Mechanisms of NETosis

As members of the first-line defence of the immune system, neutrophils are well known to interact with other cell types and active cellular crosstalk is followed by release of inflammatory mediators, stimuli-specific receptor-activation and homing. NET formation is described to occur in a particularly versatile manner under different pathophysiological conditions, and the complexity is just the beginning to be explored. We are yet to clarify which factors are required to prevent NET formation of a neutrophil and whether this alternative pro-inflammatory function of the cells can co-exist with the classical responses of the same cell. The

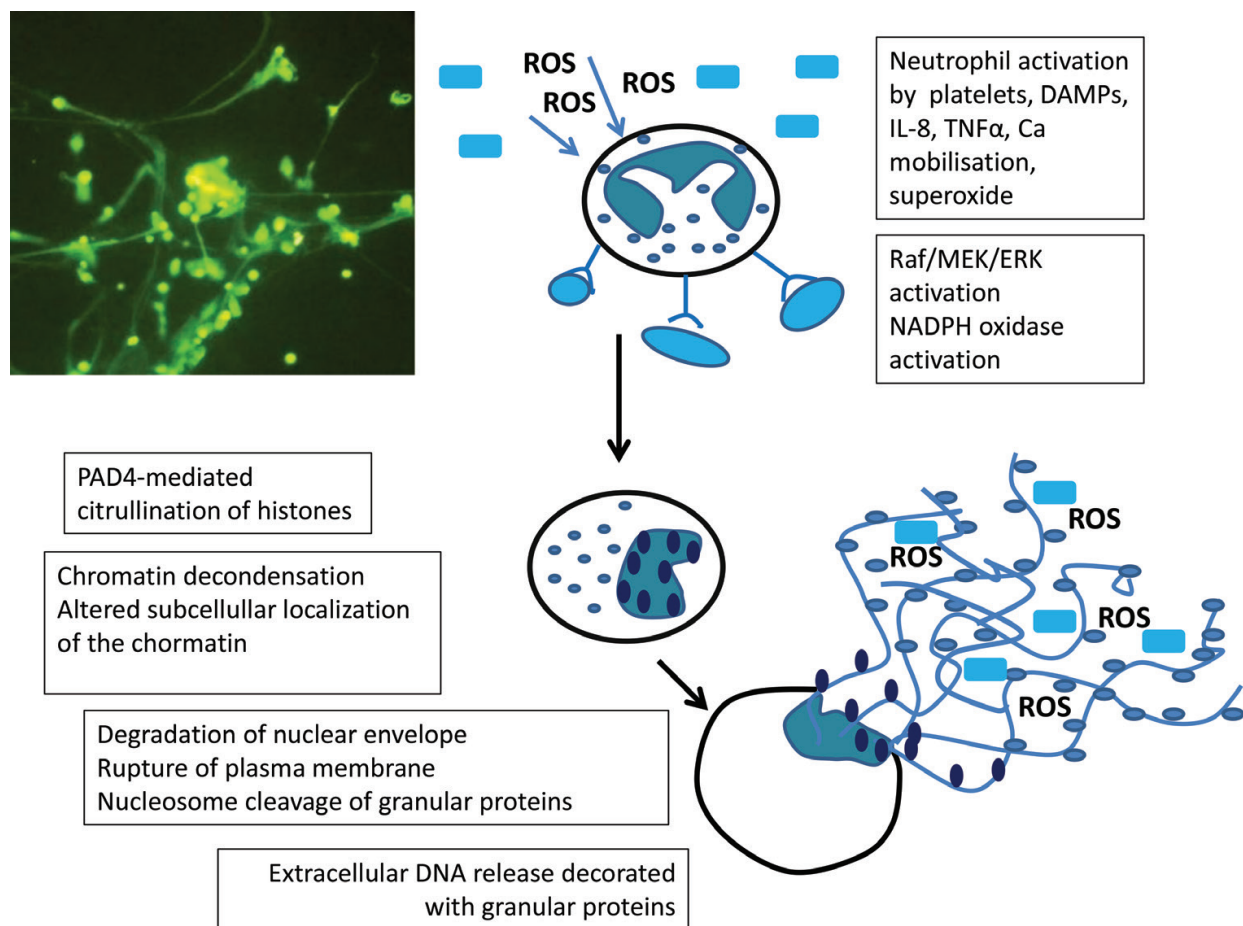


current view of the role of surrounding cells, soluble mediators and intracellular elements is overviewed below.

### 3.1. Structure and function of NETs

NETosis has been described as a process in which activated neutrophils extrude a chromatin-fibre-based meshwork encompassing their own granules and antimicrobial enzymes, such as neutrophil elastase, cathepsin G,  $\alpha$ -defensins and MPO [1]. Mass spectrometry results have revealed a series of additional protein components from various types of granules [30]. The extrinsic and intrinsic factors contributing to NET formation are summarised in **Figure 2**.

These structures represent an important strategy to immobilise and kill invading microorganisms and are considered to be evolutionarily conserved, since they target both Gram-negative and Gram-positive bacteria, viruses and fungi [31]. Besides humans, the phenomenon



**Figure 2.** (A) Representative image of neutrophils forming extracellular traps visualized by fluorescent microscopy (Nikon Diaphot 300 Inverted fluorescence & phase contrast microscope, 20 $\times$  magnification) after staining the cells with Sytox Green DNA intercalating dye. (B) Schematic figure on the possible mechanism of NET formation, where DAMP, damage-associated molecular pattern; IL-8, interleukin 8; TNF $\alpha$ , tumor necrosis factor-alpha; Raf, rapidly accelerated fibrosarcoma kinase; MEK, mitogen-activated protein kinase; ERK, extracellular signal regulated kinase; NADPH, nicotinamide adenine dinucleotide phosphate. and PAD4, protein arginine deiminase 4.

was proven to be present in insects, various vertebrates including fishes and even in plants [32–36]. The NET scaffold consists of chromatin components with a diameter of 15–17 nm and the connected proteins and microparticles. To date, nuclear DNA and histones are observed to represent the major NET constituents [1]. The exact mechanism through which the genetic material is ejected from the cell and decorated by antimicrobial factors is still not well understood. Nonetheless, it is considered to be an active process, where the cells undergo an apoptosis-like process with peptidylargininedeaminase 4 (PAD-4)-mediated DNA decondensation, membrane disintegration and chromatin realignment [37], and the role of ROS formation in the process seems to be inevitable, but the mechanism remains controversial [2].

### 3.2. Post-injury activators of NETosis

Studies aimed at describing the receptor-ligand signalling pathways are fundamental in sterile NET formation revealed diverse and sometimes controversial mechanistic details. Endogenous ligands were described to bind to TLR (mainly TLR4 and TLR9), Fc receptors (e.g. FcRIIa) or cytokine receptors (such as IL-17 R) accompanied by this process [38–40]. Complement receptor activation has also been reported to be implicated [41]. Many sterile chemical stimuli were proven to induce NETosis *in vitro* without infection such as TNF- $\alpha$ , IL-8, interferon- $\gamma$ , nicotine certain antibiotics or enhanced ROS generation produced by NADPH oxidases [1, 42–46].

As NETs consist of a significant amount of extracellular DNA as a scaffold, injury-related NET formation may cause a further elevated DAMP concentration in the circulation, and therefore, it could result in more severe tissue damage [4, 48]. Mitochondrial DNA was subsequently demonstrated to be a trigger for NETosis after major trauma and demonstrated that the signalling was mediated through a TLR9-dependent pathway, independent of the NADPH oxidase system [39]. Our group demonstrated that NETs formed after trauma were almost exclusively composed of mtDNA [4]. There has also been a relationship demonstrated in NETosis observed in systemic lupus erythematosus (SLE) where NETs released were found to be highly enriched with oxidised mtDNA [49]. Interestingly, this study also found that these NETs resulted in increased production of IFN I, which was dependent on STING pathway signalling. This perhaps suggests that mtDNA may play a role in driving autoimmunity in a rather novel and previously unstudied way.

### 3.3. Cell-cell interactions as regulators of post-injury NET formation

#### 3.3.1. Interaction with platelets

There is growing evidence on the importance of neutrophil-neutrophil crosstalk and communication with other cells related to NET formation. Platelets are far the most characterised players in NETosis as many platelet originated ligand/receptor pairs and soluble mediators perpetuate neutrophil activation [50]. The proof-of-concept *in vitro* studies demonstrated that platelet activation is crucial as the initial step [29, 51]. Human neutrophils isolated from healthy volunteers underwent a robust NET formation in the presence of activated platelets treated

with thrombin receptor-activating peptide, while no NETosis occurred with the co-incubation of resting platelets [52]. In the same study, the early event of platelet-platelet interaction was blocked with a glycoprotein IIb/IIIa inhibitor and resulted in reduced NET formation in a mice TRALI model [52]. P-selectin is suspected to largely be responsible for the ability to trigger sterile NET formation in human neutrophils [53], but other cell adhesion molecules found on platelets are demonstrated to play rather significant role as  $\beta 2$  integrin (CD18) [53, 54]. Among soluble mediators, chemokines (as CXCL4) and alarmins (as HMGB-1) produced by platelets were observed to activate neutrophils to form NETs *in vitro* and in animal models [54, 55]; however, this feature of platelets is broadly connected to any kind of inflammatory response, and therefore, the direct or indirect contribution of this phenomenon is too limited to be predictable.

### 3.3.2. Endothelium-neutrophil interactions

Circulating neutrophils tend to be quiescent and inactive, while their activation classically depends on their communication with endothelial cells. After neutrophil-endothelial interaction, the cells can rapidly undergo degranulation, activation of their NADPH oxidase system and even NET formation [56, 57]. The importance of this interface is also supported by more recent studies, where endothelium-produced matrix metalloproteinases induced NET formation followed by cytotoxicity and vessel dysfunction [58, 59].

## 3.4. Intracellular and molecular regulators of NETosis

Neutrophil extracellular trap formation is primarily dependent on histone abundance and alignment, activation of NADPH oxidase and MPO, interactions between platelets and neutrophils, expression of NET component proteins, and neutrophil autophagy.

### 3.4.1. The role of chromatin decondensation

Peptidylargininedeiminase 4 (PAD4)-mediated chromatin decondensation, which occurs in the nucleus, is apparently a critical and initial step in NET formation. PAD4 is a nuclear enzyme that converts specific arginine residues to citrulline on histone tails [60]. The release of NETs strongly depends on PAD4 activity [61] but was surprisingly found not to be essential in certain conditions [62]. Neutrophils isolated from PAD4-deficient mice were unable to citrullinate histones, decondense chromatin, and generate NETs [63]. In fact, PAD inhibitors have demonstrated efficacy in a variety of immune pathologies [64, 65], supporting the importance of this pathway in NET formation.

### 3.4.2. NADPH-dependent ROS production, Raf-MEK-ERK pathway

Hakkim and co-workers first described the importance of the Raf/MEK/ERK signalling pathway in PMA-induced NET formation and their data suggest that the Raf-MEK-ERK pathway might be upstream of NADPH oxidase activation [66]. Other studies pointed out that phosphorylation of ERK both in platelets and in neutrophils is also necessary for the formation of NETs mediated by activated platelets [52, 53].



### 3.4.3. *Toll-like receptors*

Toll-like receptors are classified according to the types of agonists that bind and the corresponding response that is activated and several of them were found to facilitate profound inflammatory responses after binding endogenous ligands [67]. It was recently reported that neutrophil stimulation via TLR activation with various molecules leads to NET production. Further to this, the structure of the NETs is characteristic to the type of TLR stimulation [68]. TLR4 seems to be responsible for this kind of neutrophil activity in particular as many publications demonstrated their interaction via HMGB-1 [55], superoxide production [69], platelet activation [29] or IL-1 $\beta$  [70]. Oxidised low-density lipoprotein, which has been implicated as an independent risk factor in various acute or chronic inflammatory diseases including SIRS, was also found to act as a NETosis trigger via TLRs [71]. More recently, TLR9 has come into focus in NET research as mtDNA and other DAMPs that are recognised by TLR9 showed high potential to induce NETs in trauma patients [39], in liver ischemia/reperfusion injury [3] or due to surgical stress [72].

## 4. Pathophysiology of post-injury NETs

### 4.1. The role of NETs in sterile inflammation

Recent discoveries have revealed the potential role of NETs in the pathogenesis of a wide range of non-infectious diseases, in particular sterile chronic inflammatory conditions such as systemic lupus erythematosus [38, 73], small vessel vasculitis [74] and psoriasis [75]. In such conditions, both spontaneous NET formation and impaired NETosis were evident. Reduced ability of PMNs for to undergo NETosis was described in diabetes mellitus patients who were exposed to bacterial infections [76] that might be a possible explanation for why this population is more susceptible to life-threatening infections. In another recent study conducted on diabetes patients, spontaneous release of isolated PMN NETs was increased, suggesting that a chronic pro-inflammatory condition during hyperglycaemia favours constitutive NET formation [77]. Chronic inflammation is also characteristic in cardiovascular diseases and indeed, NETosis was found to contribute to the pathomechanism of deep vein thrombosis [78], acute myocardial ischemia/reperfusion in a mouse model [79], and NETs were observed to be localised in limb atherosclerotic plaques [80]. Furthermore, the content of plasma MPO-DNA complexes was found to be associated with an increased risk of coronary stenosis in patients with severe coronary atherosclerosis [81]. Interestingly, healthy conditions but with an altered metabolic and oxygen consumption rate were also described to be associated with elevated NETosis of isolated PMNs. In a very recent paper, NET formation and neutrophil pro-NETotic priming were found to be augmented during the course pregnancy in healthy women when compared to matching non-pregnant control donors [82]. What was found to be elevated in the mother, seemed to be blocked in the foetus, as newborn neutrophils isolated from umbilical cord blood on the day of delivery did not form NETs when stimulated [83]. In the latter study, the authors identified a unique protein in the umbilical cord blood-called neonatal NET-inhibitory factor

(nNIF) that would raise a very interesting question of a novel foetal adaptation mechanism and therapeutic approach. Acute injuries such as AKI and ALI were both described to be relevant pathologies to study increased NETosis in humans. NET biomarkers were present in transfusion-related acute lung injury patients' blood, and in fact, NETs were produced *in vitro* by primed human neutrophils when challenged with anti-neutrophil alloantigen-3a antibodies previously implicated in TRALI [84]. In another human study, the cfDNA/NET content of 31 critically ill patient's blood was in a significant positive correlation with the severity of acute kidney injury [85]. This result encouraged the evaluation of serum (or plasma) NETs concentration as an early predictive biomarkers of complicated outcomes on the ICU.

#### 4.2. Pathophysiology of trauma-related NET formation

The potential role of NETs in the mechanical injury driven inflammatory response has recently been proposed [47, 86]. Similarly, the presence of NETs was demonstrated in a mixed intensive care unit population with systemic inflammatory response syndrome [87]. NETs have also been implicated in the pathogenesis of acute lung injury and in sterile transfusion-related acute lung injury, which are often antecedents of MOF [52]. Recently, Grimberg-Peters et al. published that neutrophils isolated from severely injured patients (days 1–2 after trauma) showed markedly elevated NET formation after pharmacological activation, and this effect was successfully attenuated by the treatment with hyperbaric oxygen [88]. This result indicates the potential importance of oxido-reductive burst in NETosis after traumatic injury, and it is well established that in such conditions, NET formation is generally NADPH oxidase-dependent [48]. However, the exact molecular mechanism behind is not fully understood, as indicated in a study by Itagakai and co-worker, where human PMNs from young and elderly trauma patients formed NETs in a great number, via TLR9 activation, but independently from NADPH oxidase activation [39].

Moreover, the DAMP release after trauma might be fundamental in further promoting NET production. Besides its role in sterile inflammation, mitochondrial DNA may have another pivotal role in worsening the inflammatory response, via NET formation. Our recent data show NETs observed after injury and subsequent surgery can be composed of mitochondrial DNA [4], and other authors have found the same phenomenon under certain conditions [89]. The exact molecular mechanism of mtDNA-NET release is unclear; however, when a ROS production inhibitor (diphenyleneiodonium) was used, mitochondrial DNA-NET formation was also blocked, and no DNA was released [89, 90].

#### 4.3. NETs as therapeutic target for post-injury inflammation

To date, the contribution of NET formation on the pathomechanism of a wide range of clinical conditions is evident, and there is emerging evidence about the potential therapeutic usefulness of pharmacological NET inhibition. While animal experiments and *in vitro* cell culture studies are promising, it is yet unknown if NET-targeting therapies can be effective in clinical practice. As many protective physiological and pathophysiological processes require NET formation, the harm/benefit ratio of NET formation inhibition is unclear.

#### 4.3.1. Chemical inhibition of NETosis

There are several drugs already used in clinical practice in autoimmune diseases that have potential for NETosis inhibition. Plaquenil Sulphate (hydroxychloroquine, HQ) is a disease-modifying anti-rheumatic drug, which inhibits prostaglandin and cytokine synthesis, and most of all induces a blockade in TLR signalling [91]. Juvenile-onset systemic lupus erythematosus patients' isolated PMNs showed augmented NET formation, which was significantly modulated with HQ treatment [92]. N-acetylcysteine (NAC), which is a commonly recommended supplement to treat various autoimmune symptoms, was described to inhibit NET release by PMA stimulated human neutrophils in a ROS-dependent manner [93]. The application of NAC had similar effect in other recently published studies [70, 94], which supports the usage of other free radical scavengers as adjuvant therapy on the ICU trauma patients. Monoclonal antibodies such as the complement inhibitor Eculizumab might open up a new perspective in drug therapies targeting NETosis based on the findings that plasma NET markers of paroxysmal nocturnal haemoglobinuria patients with thrombosis history were significantly elevated than that of controls or patients without thrombosis history, while the Eculizumab treatment normalised the values to the control level [95]. Another FDA-approved monoclonal antibody, Rituximab was also demonstrated to be protective against adverse NET formation in different human studies [96].

The inhibition of histone decondensation via PAD4 targeting of the PMNs is another potential NET-based therapeutic target, as PAD overexpression and upregulated enzyme activity have been observed in several diseases [97], and or PAD4-mediated NET formation was described to be not essential against infection [62].

The direct inhibition of the granule and protein components of NETs is another way to manipulate NET formation. However, these are essential antimicrobial peptides and mediate important physiological pathways. Currently, the literature is conflicting as to whether MPO, NE and the other compounds connected to the NET scaffold are appropriate targets. In one study, MPO-facilitated ROS-generation was proven to be required for neutrophil extracellular trap formation in humans and pharmacological inhibition of MPO delays and reduces NET formation [28, 98, 99], but recently more evidence revealed the opposite or conditional effect [100–102].

#### 4.3.2. The therapeutic effect of DNase treatment

The fact that extrachromosomal DNA and particularly mtDNA have such potent immunostimulatory effects makes it an exciting and very rational target for immunomodulation therapy and silencing NET formation is one of the many possible trends. Whether nDNA or mtDNA are conjugated with NETs, both are readily digestible with DNase. There is certainly good evidence to suggest that focally targeting NETs with DNase have yielded a reduction in associated inflammatory lung damage in a mouse model of transfusion-related acute lung injury (TRALI) [52]. Human recombinant DNase therapy has been used to good effect when nebulised in cystic fibrosis (CF) patients by enhancing sputum solubilisation [103]. This effect may be beneficial to other conditions with excessive NETosis, as several studies have recently demonstrated that NETs and NET-associated proteins are present in CF sputum [104–107].

However, there might be dangerous consequences if the extracellular DNA is not cleared up perfectly or if the freely floating pro-inflammatory peptides have entered the bloodstream. Dubois and colleagues have demonstrated that DNase administration to CF sputum dramatically increased elastase activity [108]. Thus, the combined administration of DNase and specific inhibitor could be useful to avoid the deleterious effects of excessive proteases. With such an emergent role of mtDNA in NETs associated with trauma [4] and more recently in SLE [49], the investigation of DNase therapy in different inflammatory conditions including post-injury inflammation would be very reasonable. Nevertheless, a long-term DNase therapy presents side effects to patients [109] including dramatic increase in other antimicrobial activities [108] or further impedance of the immune system which makes the host susceptible to disseminated and lethal infections [110, 111]. The latter has notable consideration in the management of major trauma patient as 39.5% of trauma deaths occur in the hospital mainly due to nosocomial infections [112].

#### 4.3.3. *The clinical predictive value of NETs*

The number of studies investigating the presence or the predictive value of NETs and NET components alongside extracellular DNA concentration as potential biomarkers in different human body fluids has grown significantly in recent years. Serum and plasma certainly are the most investigated materials, as being the natural habitat for PMNs, although it raises some concern whether activated NET-forming PMNs are representative enough in the blood.

In cases of acute injuries, such as major trauma, quantification of NETs from blood seems to be a trustworthy biomarker for clinical prediction. Margraf and co-workers published in 2008 that NETs quantities in plasma may predict multiple organ failure and sepsis on the ICU in patients after multiple trauma [12]. This ground breaking work was followed by other papers, such as the one of Altrichter and co-workers who described that circulating free-DNA neutrophil extracellular traps (cf-DNA/NETs) could be used in the prediction of mortality in a population of 32 patients with severe burn injury [13]. Similarly, early diagnosis of septic arthritis by cfDNA/NETs measurement could guide the surgical team to rescue the joint by deciding to perform an immediate operation [99]. However, in these cases, the dynamic profile of circulating neutrophils and NETs in the acute and subacute phase of inflammation should be taken into consideration when determining the optimal timing of biomarker measurement. It is also important to note that NET components, namely DNA complexes and elastase, may also accumulate in the blood during other programs of cell death, for example, during endothelial cell apoptosis or macrophage necrosis [81].

Beyond blood-based extracellular trap identification, Mohanty and co-workers described a new approach to non-invasive NET-associated biomarker research, which showed the presence of numerous neutrophils in morning saliva had undergone NETosis [113]. Tear fluid might also be informative. In a study conducted on dry eye disease (DED) patients and matching controls, tear fluid nuclease activity was decreased significantly in DED patients, whereas the amount of extracellular DNA, histones, cathelicidin, and neutrophil elastase on the ocular surface was increased significantly [114]. A similar paper characterised the activated neutrophil-specific biomarkers in the tear fluid among ocular graft versus host disease patients, and a marked increase in both NE and MPO concentrations was evident [115].



## 5. Final remarks

In this chapter, we summarised the mechanism, regulation and clinical significance of neutrophil granulocytes and the complex process of extracellular trap formation. The relevant literature shows that a highly specialised population of neutrophils facilitate NET formation in response to infection and also sterile inflammation. Interest in the potential role of NETs in the posttraumatic injury setting and their possible role in the subsequent inflammatory response has gained significant attention lately. To date, the contribution of NET formation on the pathomechanism of a wide range of clinical conditions was proven to be inevitable and the observation of NETosis became more important in post-injury clinical outcome prediction.

For the better understanding of the exact mechanistic details and the role of NETs in normal recovery and disease, improved methodology and quantification are urgently needed. The current techniques combine fluorescent microscopy or fluorescent intensity measurements and generally use DNA-intercalating dyes, while taking the risk of visualising necrotic cells with dye permeable cell membrane. Antibody-based techniques are required to detect activated, non-necrotic cells with intact cell membrane, such as flow cytometry-cell-sorting, supported by microscopic imaging. Additionally, a consensus on the structural and behavioural definition of NET formation is essential for future NET research, due to their fragility, their highly dynamic nature and their morphological heterogeneity.

## Author details

Eszter Tuboly<sup>1</sup>, Gabrielle D. Briggs<sup>2</sup> and Zsolt J. Balogh<sup>1,2\*</sup>

\*Address all correspondence to: zsolt.balogh@hnehealth.nsw.gov.au

1 Hunter New England Health, Newcastle, Australia

2 University of Newcastle, Newcastle, Australia

## References

- [1] Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. *Science*. 2004 Mar;**303**(5663):1532–1535
- [2] Kaplan MJ, Radic M. Neutrophil extracellular traps: Double-edged swords of innate immunity. *The Journal of Immunology*. 2012 Sep;**189**(6):2689–2695
- [3] Huang HS, Tohme S, Al-Khafaji AB, Tai S, Loughran P, Chen L, Wang S, Kim J, Billiar T, Wang Y, Tsung A. Damage-associated molecular pattern-activated neutrophil extracellular trap exacerbates sterile inflammatory liver injury. *Hepatology*. 2015 Aug;**62**(2):600–614



- [4] McIlroy DJ, Jarnicki AG, Au GG, Lott N, Smith DW, Hansbro PM, Balogh ZJ. Mitochondrial DNA neutrophil extracellular traps are formed after trauma and subsequent surgery. *Journal of Critical Care*. 2014 Dec;**29**(6):1133.e1–e5
- [5] Leliefeld PH, Wessels CM, Leenen LP, Koenderman L, Pillay J. The role of neutrophils in immune dysfunction during severe inflammation. *Critical Care*. 2016 Mar;**20**:73
- [6] Klein Klouwenberg PM, Ong DS, Bonten MJ, Cremer OL. Classification of sepsis, severe sepsis and septic shock: The impact of minor variations in data capture and definition of SIRS criteria. *Intensive Care Medicine*. 2012 May;**38**(5):811–819
- [7] Osborn TM, Tracy JK, Dunne JR, Pasquale M, Napolitano LM. Epidemiology of sepsis in patients with traumatic injury. *Critical Care Medicine*. 2004 Nov;**32**(11):2234–2240
- [8] Lazarus HM, Fox J, Burke JP, Lloyd JF, Snow GL, Mehta RR, Evans RS, Abouzelof R, Taylor C, Stevens MH. Trauma patient hospital-associated infections: Risks and outcomes. *Journal of Trauma and Acute Care Surgery*. 2005 Jul;**59**(1):188–194
- [9] Gutierrez T, Hornigold R, Pearce A. The systemic response to surgery. *Surgery*. 2014 March;**32**(3):149–152
- [10] Glance LG, Stone PW, Mukamel DB, Dick AW. Increases in mortality, length of stay, and cost associated with hospital-acquired infections in trauma patients. *The Art and Science of Surgery*. 2011 Jul;**146**(7):794–801
- [11] Hietbrink F, Koenderman L, Rijkers G, Leenen L. Trauma: The role of the innate immune system. *World Journal of Emergency Surgery*. 2006 May **20**:1:15
- [12] Dewar D, Moore FA, Moore EE, Balogh Z. Postinjury multiple organ failure. *Injury*. 2009 Sep;**40**(9):912–918
- [13] Margraf S, Lögters T, Reipen J, Altrichter J, Scholz M, Windolf J. Neutrophil-derived circulating free DNA (cf-DNA/NETs): A potential prognostic marker for posttraumatic development of inflammatory second hit and sepsis. *Shock*. 2008 Oct;**30**(4):352–358
- [14] Altrichter J, Zedler S, Kraft R, Faist E, Mitzner SR, Sauer M, Windolf J, Scholz M, Lögters T. Neutrophil-derived circulating free DNA (cf-DNA/NETs), a potential prognostic marker for mortality in patients with severe burn injury. *European Journal of Trauma and Emergency Surgery*. 2010 Dec;**36**(6):551–557
- [15] Brinkmann V, Zychlinsky A. Neutrophil extracellular traps: Is immunity the second function of chromatin? *The Journal of Cell Biology*. 2012 Sep;**198**(5):773–783
- [16] Tibrewal S, Ivanir Y, Sarkar J, Nayeb-Hashemi N, Bouchard CS, Kim E, Jain S. Hyperosmolar stress induces neutrophil extracellular trap formation: Implications for dry eye disease. *Investigative Ophthalmology & Visual Science*. 2014 Nov;**55**(12):7961–7969
- [17] Helseth R, Solheim S, Arnesen H, Seljeflot I, Opstad TB. The time course of markers of neutrophil extracellular traps in patients undergoing revascularization for acute myocardial infarction or stable angina pectoris. *Mediators of Inflammation*. 2016;**2016**:2182358

- [18] Keel M, Trentz O. Pathophysiology of polytrauma. *Injury*. 2005 Jun;**36**(6):691–709
- [19] Ward PA, Lentsch AB. The acute inflammatory response and its regulation. *Archives of Surgery*. 1999 Jun;**134**(6):666–669
- [20] Bone RC. Immunologic dissonance: A continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). *Annals of Internal Medicine*. 1996 Oct;**125**(8):680–687
- [21] Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines—CXC and CC chemokines. *Advances in Immunology*. 1994;**55**:97–179
- [22] Dinarello CA. Role of pro- and anti-inflammatory cytokines during inflammation: Experimental and clinical findings. *Journal of Biological Regulators & Homeostatic Agents*. 1997 Jul-Sep;**11**(3):91–103
- [23] Feghali CA, Wright TM. Cytokines in acute and chronic inflammation. *Frontiers in Bioscience*. 1997 Jan;**2**:d12–d26
- [24] Trzeciak S, Dellinger RP, Parrillo JE, Guglielmi M, Bajaj J, Abate NL, Arnold RC, Colilla S, Zanoiti S, Hollenberg SM. Microcirculatory alterations in resuscitation and shock investigators. *Annals of Emergency Medicine*. 2007 Jan;**49**(1):88–98, 98.e1–e2
- [25] Kunkel SL, Strieter RM, Lindley IJ, Westwick J. Chemokines: New ligands, receptors and activities. *Immunology Today*. 1995 Dec;**16**(12):559–561
- [26] Wen H, Dou Y, Hogaboam CM, Kunkel SL. Epigenetic regulation of dendritic cell-derived interleukin-12 facilitates immunosuppression after a severe innate immune response. *Blood*. 2008 Feb;**111**(4):1797–1804
- [27] Bataille A, Galichon P, Zilietis MJ, Sadia I, Hertig A. Epigenetic changes during sepsis: On your marks! *Critical Care*. 2015 Oct;**19**:358
- [28] Brinkmann V, Laube B, Abu Abed U, Goosmann C, Zychlinsky A. Neutrophil extracellular traps: How to generate and visualize them. *Journal of Visualized Experiments*. 2010 Feb;**(36)**:1724
- [29] Metzler KD, Fuchs TA, Nauseef WM, Reumaux D, Roesler J, Schulze I, Wahn V, Papayannopoulos V, Zychlinsky A. Myeloperoxidase is required for neutrophil extracellular trap formation: Implications for innate immunity. *Blood*. 2011 Jan;**117**(3):953–959
- [30] 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 FH, Kubes P. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nature Medicine*. 2007 Apr;**13**(4):463–469
- [31] Jaillon S, Peri G, Delneste Y, Frémaux I, Doni A, Moalli F, Garlanda C, Romani L, Gascan H, Bellocchio S, Bozza S, Cassatella MA, Jeannin P, Mantovani A. The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. *The Journal of Experimental Medicine*. 2007 Apr;**204**(4):793–804

- [32] Urban CF, Lourido S, Zychlinsky A. How do microbes evade neutrophil killing? *Cellular Microbiology*. 2006 Nov;**8**(11):1687–1696
- [33] Palić D, Ostojić J, Andreasen CB, Roth JA. Fish cast NETs: Neutrophil extracellular traps are released from fish neutrophils. *Developmental & Comparative Immunology*. 2007;**31**(8):805–816
- [34] Altincicek B, Stötzl S, Wygrecka M, Preissner KT, Vilcinskas A. Host-derived extracellular nucleic acids enhance innate immune responses, induce coagulation, and prolong survival upon infection in insects. *The Journal of Immunology*. 2008 Aug;**181**(4):2705–2712
- [35] Ermert D1, Urban CF, Laube B, Goosmann C, Zychlinsky A, Brinkmann V. Mouse neutrophil extracellular traps in microbial infections. *Journal of Innate Immunity*. 2009;**1**(3):181–193
- [36] Wen F, White GJ, VanEtten HD, Xiong Z, Hawes MC. Extracellular DNA is required for root tip resistance to fungal infection. *Plant Physiology*. 2009 Oct;**151**(2):820–829
- [37] Wardini AB, Guimarães-Costa AB, Nascimento MT, Nadaes NR, Danelli MG, Mazur C, Benjamim CF, Saraiva EM, Pinto-da-Silva LH. Characterization of neutrophil extracellular traps in cats naturally infected with feline leukemia virus. *Journal of General Virology*. 2010 Jan;**91**(Pt 1):259–264
- [38] Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, Weinrauch Y, Brinkmann V, Zychlinsky A. Novel cell death program leads to neutrophil extracellular traps. *Journal of Cell Biology*. 2007 Jan;**176**(2):231–241
- [39] Garcia-Romo GS, Caielli S, Vega B, Connolly J, Allantaz F, Xu Z, Punaro M, Baisch J, Guiducci C, Coffman RL, Barrat FJ, Banchereau J, Pascual V. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Science Translational Medicine*. 2011 Mar;**3**(73):73ra20
- [40] Itagaki K, Kaczmarek E, Lee YT, Tang IT, Isal B, Adibnia Y, Sandler N, Grimm MJ, Segal BH, Otterbein LE, Hauser CJ. Mitochondrial DNA released by trauma induces neutrophil extracellular traps. *PLoS One*. 2015 Mar;**10**(3):e0120549
- [41] Suzuki E, Maverakis E, Sarin R, Bouchareychas L, Kuchroo VK, Nestle FO, Adamopoulos IE. T Cell-independent mechanisms associated with neutrophil extracellular trap formation and selective autophagy in IL-17A-mediated epidermal hyperplasia. *The Journal of Immunology*. 2016 Dec;**197**(11):4403–4412
- [42] Munks MW, McKee AS, Macleod MK, Powell RL, Degen JL, Reisdorph NA, Kappler JW, Marrack P. Aluminum adjuvants elicit fibrin-dependent extracellular traps in vivo. *Blood*. 2010 Dec;**116**(24):5191–5199
- [43] Keshari RS, Jyoti A, Dubey M, Kothari N, Kohli M, Bogra J, Barthwal MK, Dikshit M. Cytokines induced neutrophil extracellular traps formation: Implication for the inflammatory disease condition. *PLoS One*. 2012;**7**(10):e48111

- [44] Björnsdóttir H1, Granfeldt D, Welin A, Bylund J, Karlsson A. Inhibition of phospholipase A(2) abrogates intracellular processing of NADPH-oxidase derived reactive oxygen species in human neutrophils. *Experimental Cell Research*. 2013 Mar;**319**(5):761–774
- [45] Zawrotniak M, Rapala-Kozik M. Neutrophil extracellular traps (NETs)—Formation and implications. *Acta Biochimica Polonica*. 2013;**60**(3):277–884
- [46] Hosseinzadeh A, Thompson PR, Segal BH, Urban CF. Nicotine induces neutrophil extracellular traps. *Journal of Leukocyte Biology*. 2016 Nov;**100**(5):1105–1112
- [47] Manda-Handzlik A, Bystrzycka W, Sieczkowska S, Demkow U, Ciepiela O. Antibiotics modulate the ability of neutrophils to release neutrophil extracellular traps. *Advances in Experimental Medicine and Biology*. 20 Sep 2016. [Epub ahead of print]
- [48] Liu FC, Chuang YH, Tsai YF, Yu HP. Role of neutrophil extracellular traps following injury. *Shock*. 2014 Jun;**41**(6):491–498
- [49] Almyroudis NG, Grimm MJ, Davidson BA, Röhm M, Urban CF, Segal BH. NETosis and NADPH oxidase: At the intersection of host defense, inflammation, and injury. *Frontiers in Immunology*. 2013 Mar;**4**:45
- [50] Lood C, Blanco LP, Purmalek MM, Carmona-Rivera C, De Ravin SS, Smith CK, Malech HL, Ledbetter JA, Elkon KB, Kaplan MJ. Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nature Medicine*. 2016 Feb;**22**(2):146–153
- [51] Kazzaz NM, Sule G, Knight JS. Intercellular interactions as regulators of NETosis. *Frontiers in Immunology*. 2016;**7**:453. eCollection 2016
- [52] Maugeri N, Campana L, Gavina M, Covino C, De Metrio M, Panciroli C, Maiuri L, Maseri A, D'Angelo A, Bianchi ME, Rovere-Querini P, Manfredi AA. Activated platelets present high mobility group box 1 to neutrophils, inducing autophagy and promoting the extrusion of neutrophil extracellular traps. *Journal of Thrombosis and Haemostasis*. 2014 Dec;**12**(12):2074–2088
- [53] Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, Toy P, Werb Z, Looney MR. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *Journal of Clinical Investigation*. 2012 Jul;**122**(7):2661–2671
- [54] Carestia A, Kaufman T, Schattner M. Platelets: New bricks in the building of neutrophil extracellular traps. *Frontiers in Immunology*. 2016 Jul;**7**:271
- [55] Rossaint J, Herter JM, Van Aken H, Napirei M, Döring Y, Weber C, Soehnlein O, Zarbock A. Synchronized integrin engagement and chemokine activation is crucial in neutrophil extracellular trap-mediated sterile inflammation. *Blood*. 2014 Apr;**123**(16):2573–2584
- [56] Tadie JM, Bae HB, Jiang S, Park DW, Bell CP, Yang H, Pittet JF, Tracey K, Thannickal VJ, Abraham E, Zmijewski JW. HMGB1 promotes neutrophil extracellular trap formation through interactions with Toll-like receptor 4. *American Journal of Physiology*. 2013 Mar;**304**(5):L342–L349



- [57] Guthrie LA, McPhail LC, Henson PM, Johnston RB Jr. Priming of neutrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide. Evidence for increased activity of the superoxide-producing enzyme. *The Journal of Experimental Medicine*. 1984 Dec;**160**(6):1656–1671
- [58] Williams MR, Azcutia V, Newton G, Alcaide P, Luscinskas FW. Emerging mechanisms of neutrophil recruitment across endothelium. *Trends in Immunology*. 2011 Oct;**32**(10):461–469
- [59] Gupta AK, 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 Letters*. 2010 Jul;**584**(14):3193–3197
- [60] 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**(2):e32366
- [61] Arita K, Shimizu T, Hashimoto H, Hidaka Y, Yamada M, Sato M. Structural basis for histone N-terminal recognition by human peptidylargininedeiminase 4. *Proceedings of the National Academy of Sciences*. 2006 Apr;**103**(14):5291–5296
- [62] Rohrbach AS, Slade DJ, Thompson PR, Mowen KA. Activation of PAD4 in NET formation. *Frontiers in Immunology*. 2012 Nov ;**3**:360
- [63] Hemmers S, Teijaro JR, Arandjelovic S, Mowen KA. PAD4-mediated neutrophil extracellular trap formation is not required for immunity against influenza infection. *PLoS One*. 2011;**6**(7):e22043
- [64] Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. *Journal of Experimental Medicine*. 2010 Aug;**207**(9):1853–1862
- [65] Chumanevich AA1, Causey CP, Knuckley BA, Jones JE, Poudyal D, Chumanevich AP, Davis T, Matesic LE, Thompson PR, Hofseth LJ. Suppression of colitis in mice by Cl-amidine: A novel peptidylargininedeiminase inhibitor. *American Journal of Physiology. Gastrointestinal and Liver Physiology*. 2011 Jun;**300**(6):G929–G938
- [66] Willis VC, Gizinski AM, Banda NK, Causey CP, Knuckley B, Cordova KN, Luo Y, Levitt B, Glogowska M, Chandra P, Kulik L, Robinson WH, Arend WP, Thompson PR, Holers VM. N- $\alpha$ -benzoyl-N5-(2-chloro-1-iminoethyl)-L-ornithine amide, a protein arginine deiminase inhibitor, reduces the severity of murine collagen-induced arthritis. *The Journal of Immunology*. 2011 Apr;**186**(7):4396–4404
- [67] Hakkim A, Fuchs TA, Martinez NE, Hess S, Prinz H, Zychlinsky A, Waldmann H. Activation of the Raf-MEK-ERK pathway is required for neutrophil extracellular trap formation. *Nature Chemical Biology*. 2011 Feb;**7**(2):75–77
- [68] Holloway TL, Nicholson SE, Rani M, Cap AP, Schwacha MG. Toll-like receptor responses are suppressed in trauma ICU patients. *Journal of Surgical Research*. 2016 Nov;**206**(1):139–145



- [69] Singh RR, Randhawa D, Valera I. Human neutrophil extracellular trap formation is modulated upon stimulation with Toll-like-receptor ligands. *The Journal of Immunology*. 2016 May; **196** (1 Supplement): 60.7
- [70] Al-Khafaji AB, Tohme S, Yazdani HO, Miller D, Huang H, Tsung A. Superoxide induces neutrophil extracellular trap formation in a TLR-4 and NOX-dependent mechanism. *Molecular Medicine*. 2016 Jul; **22**:621–631
- [71] Tashiro M, Sasatomi Y, Watanabe R, Watanabe M, Miyake K, Abe Y, Yasuno T, Ito K, Ueki N, Hamauchi A, Noda R, Hisano S, Nakashima H. IL-1 $\beta$  promotes tubulointerstitial injury in MPO-ANCA-associated glomerulonephritis. *Clinical Nephrology*. 2016 Oct; **86**(10):190–199
- [72] Awasthi D, Nagarkoti S, Kumar A, Dubey M, Singh AK, Pathak P, Chandra T, Barthwal MK, Dikshit M. Oxidized LDL induced extracellular trap formation in human neutrophils via TLR-PKC-IRAK-MAPK and NADPH-oxidase activation. *Free Radical Biology & Medicine*. 2016 Apr; **93**:190–203
- [73] 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 Research*. 2016 Mar; **76**(6):1367–1380
- [74] Villanueva E, Yalavarthi S, Berthier CC, Hodgins JB, Khandpur R, Lin AM, Rubin CJ, Zhao W, Olsen SH, Klinker M, Shealy D, Denny MF, Plumas J, Chaperot L, Kretzler M, Bruce AT, Kaplan MJ. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. *The Journal of Immunology*. 2011 Jul; **187**(1):538–552
- [75] Nakazawa D, Tomaru U, Suzuki A, Masuda S, Hasegawa R, Kobayashi T, Nishio S, Kasahara M, Ishizu A. Abnormal conformation and impaired degradation of propylthiouracil-induced neutrophil extracellular traps: Implications of disordered neutrophil extracellular traps in a rat model of myeloperoxidase antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis & Rheumatology*. 2012 Nov; **64**(11):3779–3787
- [76] Lin AM, Rubin CJ, Khandpur R, Wang JY, Riblett M, Yalavarthi S, Villanueva EC, Shah P, Kaplan MJ, Bruce AT. Mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis. *The Journal of Immunology*. 2011 Jul; **187**(1):490–500
- [77] Riyapa D, Buddhisa S, Korbsrisate S, Cuccui J, Wren BW, Stevens MP, Ato M, Lertmemongkolkchai G. Neutrophil extracellular traps exhibit antibacterial activity against *Burkholderia pseudomallei* and are influenced by bacterial and host factors. *Infection and Immunity*. 2012 Nov; **80**(11):3921–3929
- [78] Joshi MB, Lad A, Bharath Prasad AS, Balakrishnan A, Ramachandra L, Satyamoorthy K. High glucose modulates IL-6 mediated immune homeostasis through impeding neutrophil extracellular trap formation. *FEBS Letters*. 2013 Jul; **587**(14):2241–2246
- [79] Fuchs TA, Brill A, Wagner DD. Neutrophil extracellular trap (NET) impact on deep vein thrombosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2012 Aug; **32**(8):1777–1783

- [80] Savchenko AS, Borissoff JL, Martinod K, De Meyer SF, Gallant M, Erpenbeck L, Brill A, Wang Y, Wagner DD. VWF-mediated leukocyte recruitment with chromatin decondensation by PAD4 increases myocardial ischemia/reperfusion injury in mice. *Blood*. 2014 Jan;**123**(1):141–148
- [81] Quillard T, Araújo HA, Franck G, Shvartz E, Sukhova G, Libby P. TLR2 and neutrophils potentiate endothelial stress, apoptosis and detachment: Implications for superficial erosion. *European Heart Journal*. 2015 Jun;**36**(22):1394–1404.
- [82] Döring Y, Weber C, Soehnlein O. Footprints of neutrophil extracellular traps as predictors of cardiovascular risk. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2013 Aug;**33**(8):1735–1736
- [83] Giaglis S, Stoikou M, Sur Chowdhury C, Schaefer G, Grimolizzi F, Rossi SW, Hoesli IM, Lapaire O, Hasler P, Hahn S. Multimodal regulation of NET formation in pregnancy: Progesterone antagonizes the Pro-NETotic effect of estrogen and G-CSF. *Frontiers in Immunology*. 2016 Dec;**7**:565
- [84] Yost CC, Schwartz H, Cody MJ, Wallace JA, Campbell RA, Vieira-de-Abreu A, Araujo CV, Schubert S, Harris ES, Rowley JW, Rondina MT, Fulcher JM, Koenig CL, Weyrich AS, Zimmerman GA. Neonatal NET-inhibitory factor and related peptides inhibit neutrophil extracellular trap formation. *Journal of Clinical Investigation*. 2016 Oct;**126**(10):3783–3798
- [85] Thomas GM, Carbo C, Curtis BR, Martinod K, Mazo IB, Schatzberg D, Cifuni SM, Fuchs TA, von Andrian UH, Hartwig JH, Aster RH, Wagner DD. Extracellular DNA traps are associated with the pathogenesis of TRALI in humans and mice. *Blood*. 2012 Jun;**119**(26):6335–6343
- [86] Czaikoski PG, Mota JM, Nascimento DC, Sônego F, Castanheira FV, Melo PH, Scortegagna GT, Silva RL, Barroso-Sousa R, Souto FO, Pazin-Filho A, Figueiredo F, Alves-Filho JC, Cunha FQ, Balogh ZJ, McIlroy DJ, Smith DW, Hansbro PM. The origin and the role of mitochondrial DNA in postinjury inflammation. *Journal of Critical Care*. 2013 Dec;**28**(6):1099–1100
- [87] Hamaguchi S, Hirose T, Akeda Y, Matsumoto N, Irisawa T, Seki M, Hosotsubo H, Tasaki O, Oishi K, Shimazu T, Tomono K. Identification of neutrophil extracellular traps in the blood of patients with systemic inflammatory response syndrome. *Journal of International Medical Research*. 2013 Feb;**41**(1):162–168
- [88] Grimberg-Peters D, Büren C, Windolf J, Wahlers T, Paunel-Görgülü A. Hyperbaric oxygen reduces production of reactive oxygen species in neutrophils from polytraumatized patients yielding in the inhibition of p38 MAP Kinase and downstream pathways. *PLoS One*. 2016 Aug;**11**(8):e0161343
- [89] Yousefi S, Mihalache C, Kozłowski E, Schmid I, Simon HU. Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death & Differentiation*. 2009 Nov;**16**(11):1438–1444

- [90] Yousefi S, Simon D, Simon HU. Eosinophil extracellular DNA traps: Molecular mechanisms and potential roles in disease. *Current Opinion in Immunology*. 2012 Dec;**24**(6):736–739
- [91] Lee SJ, Silverman E, Bargman JM. The role of antimalarial agents in the treatment of SLE and lupus nephritis. *Nature Reviews Nephrology*. 2011 Oct;**7**(12):718–729
- [92] Makuloluwa KK, Midgley A, Beresford M. Neutrophil extracellular trap-mediated activation of endosomal toll-like receptors induce immune activation in juvenile-onset systemic lupus erythematosus. *Pediatric Rheumatology*. 2014;**12**(Suppl 1):P110
- [93] Kirchner T, Hermann E, Möller S, Klinger M, Solbach W, Laskay T, Behnen M. Flavonoids and 5-aminosalicylic acid inhibit the formation of neutrophil extracellular traps. *Mediators of Inflammation*. 2013;**2013**:710239
- [94] Rada B, Jendrysik MA, Pang L, Hayes CP, Yoo DG, Park JJ, Moskowitz SM, Malech HL, Leto TL. Pyocyanin-enhanced neutrophil extracellular trap formation requires the NADPH oxidase. *PLoS One*. 2013;**8**(1):e54205
- [95] vanBijnen ST, Wouters D, van Mierlo GJ, Muus P, Zeerleder S. Neutrophil activation and nucleosomes as markers of systemic inflammation in paroxysmal nocturnal hemoglobinuria: Effects of eculizumab. *Journal of Thrombosis and Haemostasis*. 2015 Nov;**13**(11):2004–2011
- [96] Gupta S, Kaplan MJ. The role of neutrophils and NETosis in autoimmune and renal diseases. *Nature Reviews Nephrology*. 2016 Jul;**12**(7):402–413
- [97] Bicker KL, Thompson PR. The protein arginine deiminases: Structure, function, inhibition, and disease. *Biopolymers*. 2013 Feb;**99**(2):155–163
- [98] Lögters T, Margraf S, Altrichter J, Cinatl J, Mitzner S, Windolf J, Scholz M. The clinical value of neutrophil extracellular traps. *Medical Microbiology and Immunology*. 2009 Nov;**198**(4):211–219
- [99] Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *The Journal of Cell Biology*. 2010 Nov;**191**(3):677–691
- [100] 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. *Journal of Leukocyte Biology*. 2012 Oct;**92**(4):841–849
- [101] Rochael NC, Guimarães-Costa AB, Nascimento MT, DeSouza-Vieira TS, Oliveira MP, Garcia e Souza LF, Oliveira MF, Saraiva EM. Classical ROS-dependent and early/rapid ROS-independent release of neutrophil extracellular traps triggered by *Leishmania* parasites. *Scientific Reports*. 2015 Dec;**5**:18302
- [102] Martinod K, Witsch T, Farley K, Gallant M, Remold-O'Donnell E, Wagner DD. Neutrophil elastase-deficient mice form neutrophil extracellular traps in an experimental model of deep vein thrombosis. *Journal of Thrombosis and Haemostasis*. 2016 Mar;**14**(3):551–558

- [103] Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsey BW, Rosenstein BJ, Smith AL, Wohl ME. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. The New England Journal of Medicine. 1994 Sep;**331**(10):637–642
- [104] Marcos V, Zhou Z, Yildirim AO, Bohla A, Hector A, Vitkov L, Wiedenbauer EM, Krautgartner WD, Stoiber W, Belohradsky BH, Rieber N, Kormann M, Koller B, Roscher A, Roos D, Griesse M, Eickelberg O, Döring G, Mall MA, Hartl D. CXCR2 mediates NADPH oxidase-independent neutrophil extracellular trap formation in cystic fibrosis airway inflammation. Nature Medicine. 2010 Sep;**16**(9):1018–1023
- [105] Manzenreiter R, Kienberger F, Marcos V, Schilcher K, Krautgartner WD, Obermayer A, Huml M, Stoiber W, Hector A, Griesse M, Hannig M, Studnicka M, Vitkov L, Hartl D. Ultrastructural characterization of cystic fibrosis sputum using atomic force and scanning electron microscopy. Journal of Cystic Fibrosis. 2012 Mar;**11**(2):84–92
- [106] Dwyer M, Shan Q, D’Ortona S, Maurer R, Mitchell R, Olesen H, Thiel S, Huebner J, Gadjeva M. Cystic fibrosis sputum DNA has NETosis characteristics and neutrophil extracellular trap release is regulated by macrophage migration-inhibitory factor. Journal of Innate Immunity. 2014;**6**(6):765–779
- [107] Yoo DG, Floyd M, Winn M, Moskowitz SM, Rada B. NET formation induced by *Pseudomonas aeruginosa* cystic fibrosis isolates measured as release of myeloperoxidase-DNA and neutrophil elastase-DNA complexes. Immunology Letters. 2014 Aug;**160**(2):186–194. DOI: 10.1016/j.imlet.2014.03.003. Epub23Mar2014
- [108] Dubois AV, Gauthier A, Bréa D, Varaigne F, Diot P, Gauthier F, Attucci S. Influence of DNA on the activities and inhibition of neutrophil serine proteases in cystic fibrosis sputum. American Journal of Respiratory Cell and Molecular Biology. 2012 Jul;**47**(1):80–86
- [109] Porto BN, Stein RT. Neutrophil extracellular traps in pulmonary diseases: Too much of a good thing? Frontiers in Immunology. 2016 Aug;**7**:311
- [110] Beiter K, Wartha F, Albiger B, Normark S, Zychlinsky A, Henriques-Normark B. An endonuclease allows *Streptococcus pneumoniae* to escape from neutrophil extracellular traps. Current Biology. 2006 Feb;**16**(4):401–407
- [111] Meng W, Paunel-Görgülü A, Flohé S, Hoffmann A, Witte I, MacKenzie C, Baldus SE, Windolf J, Lögters TT. Depletion of neutrophil extracellular traps in vivo results in hypersusceptibility to polymicrobial sepsis in mice. Critical Care. 2012 Jul;**16**(4):R137
- [112] Jin H, Liu Z, Xiao Y, Fan X, Yan J, Liang H. Prediction of sepsis in trauma patients. Burns & Trauma. 2014 Jul;**2**(3):106–113
- [113] Mohanty T, Sjögren J, Kahn F, Abu-Humaidan AH, Fisker N, Assing K, Mörgelin M, Bengtsson AA, Borregaard N, Sørensen OE. A novel mechanism for NETosis provides antimicrobial defense at the oral mucosa. Blood. 2015 Oct;**126**(18):2128–2137

- [114] Sonawane S, Khanolkar V, Namavari A, Chaudhary S, Gandhi S, Tibrewal S, Jassim SH, Shaheen B, Hallak J, Horner JH, Newcomb M, Sarkar J, Jain S. Ocular surface extracellular DNA and nuclease activity imbalance: A new paradigm for inflammation in dry eye disease. *Investigative Ophthalmology & Visual Science*. 2012 Dec;53(13):8253–8263
- [115] Arafat SN, Robert MC, Abud T, Spurr-Michaud S, Amparo F, Dohlman CH, Dana R, Gipson IK. Elevated neutrophil elastase in tears of ocular graft-versus-host disease patients. *American Journal of Ophthalmology*. 2017 Jan. pii: S0002–S9394(17)30002–8